Volume 7, No. 1 | 2013

CCR connections

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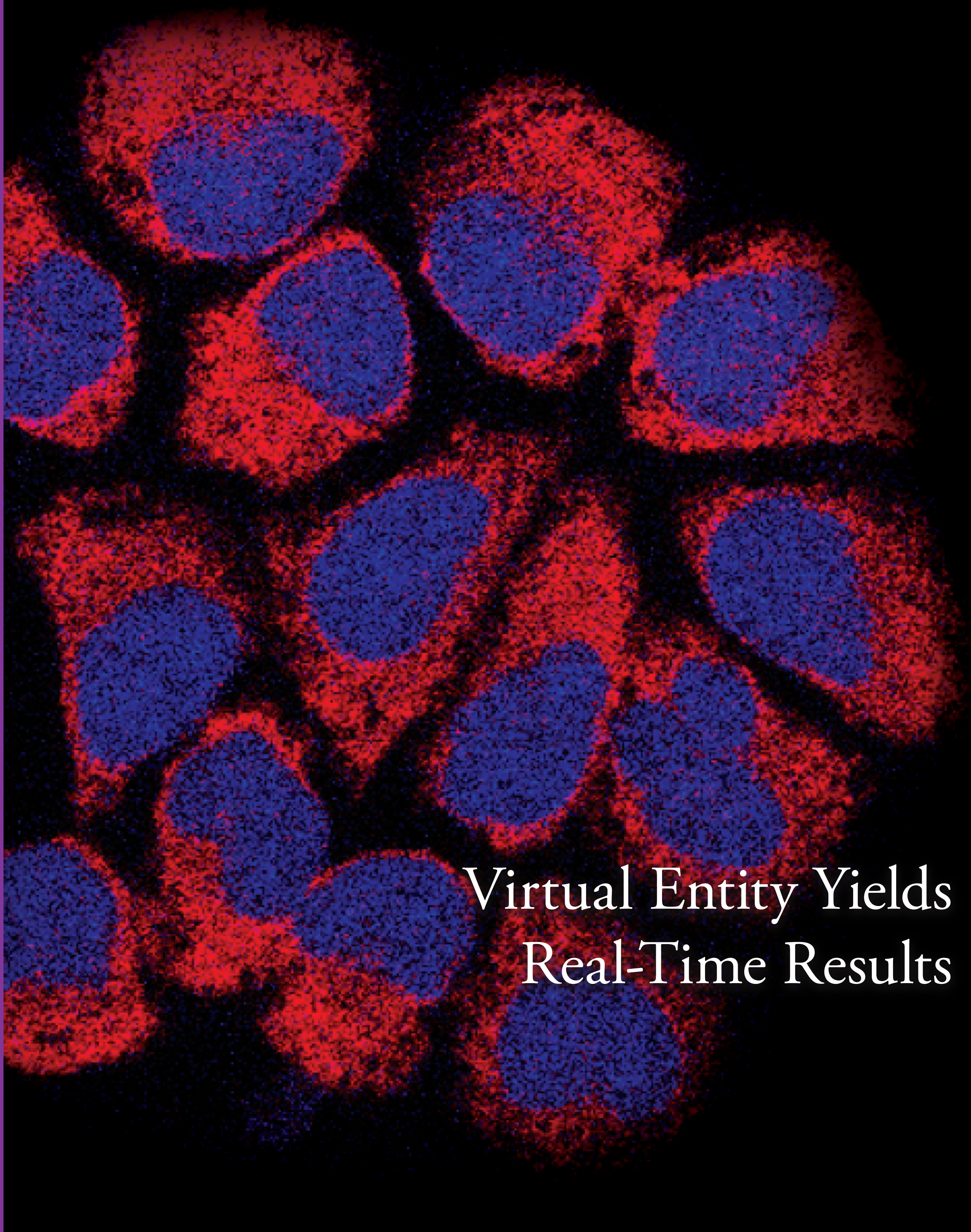
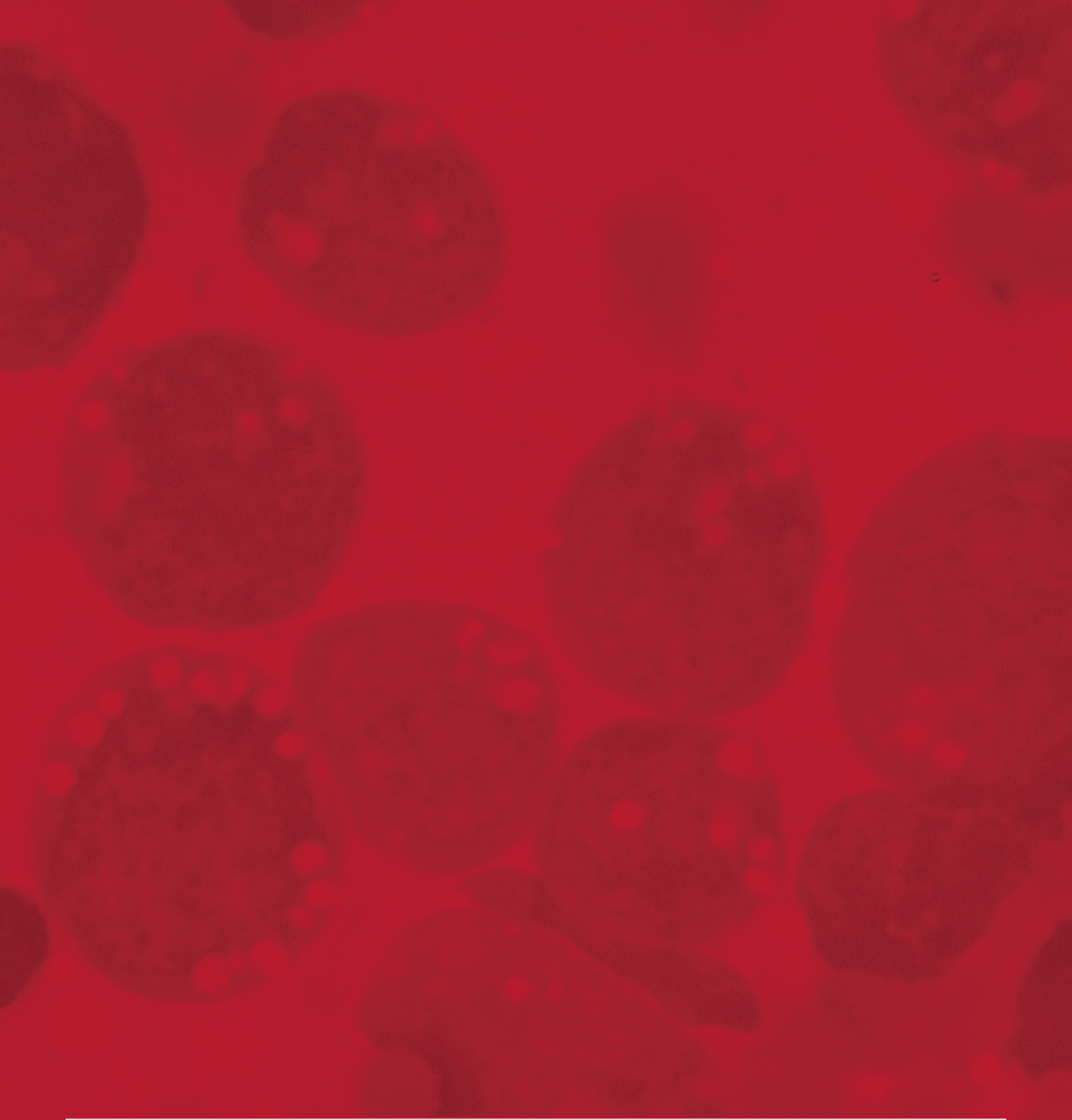


Virtual Entity Yields Real-Time Results

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health



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CENTER FOR CANCER RESEARCH

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The mission of CCR is:

To inform and empower the entire cancer research community by making breakthrough discoveries in basic and clinical cancer research and by developing them into novel therapeutic interventions for adults and children afflicted with cancer or infected with HIV.

http://home.ccr.cancer.gov/connections

Producing Scientific Synergism

Cancer researchers have long recognized the value of interdisciplinary research and collaboration because difficult scientific problems often cut across the borders of individual focus areas and disciplines. And as cancer's complexity becomes more evident, so does the need to increase the synergism produced by interdisciplinary teamwork. Accordingly, at CCR, we work assiduously to promote opportunities, including training, that bring basic and clinical researchers side by side, where they team to make important advances. Examples abound at CCR that validate this approach.

In this issue, we showcase how CCR's basic researchers generate discoveries with downstream relevance for better patient care. In "It Starts with a Choice," we meet Mirit Aladjem, Ph.D., who is elucidating the fundamental details behind DNA replication in both cancerous and normal cells. Aladjem explains that cancer cells burdened by unstable DNA face a critical option: They can replicate their DNA, pass through mitosis, and potentially initiate tumors, or they can self-destruct through apoptosis. Her research is now showing that interfering with replication machinery, such as the "replication origins" that coordinate DNA replication in cancer cells, may provide a new target for cancer therapy.

In CCR's interdisciplinary setting, clinical advances rely on basic science, and basic scientists rely on clinical observations to inform their research. In "Virtual Entity Yields Real-Time Results," we see how CCR scientists working through the Center of Excellence in Integrative Cancer Biology and Genomics (CEICBG) pool their knowledge, tools, and technologies of their separate disciplines to investigate cancer broadly, from tumor biology to potential therapies. Investigators in CEICBG identify genetic features that may be targeted in cancer drug development or used as biomarkers to predict patient responses to treatments. Their discoveries provide insights into cancer risk and progression. For example, their studies looking at somatic copy number alterations have identified "driver" mutations that promote tumor growth and may even lead to new diagnostic methods for staging liver cancer. Other ongoing studies are investigating genetic variants that could play a role in global health disparities for lung cancers. Still other research teams are identifying biomarkers to detect noncancerous pediatric cancers that are likely to transition to aggressive cancers. Cumulatively, these studies may make it possible to better match the right treatment to the right patient.

(Photo: B. Branson)

Robert Wiltrout, Ph.D.

CCR's emphasis on interdisciplinary training enriches the broader oncology research community because many who study here ultimately leave and assume leadership positions in their respective fields. In "Training the Next Generation of Cancer Researchers," we explore the selfless contributions of our principal investigators who adopt young investigators-in-training and prepare them for their future successes.

Promoting and managing a culture of interdisciplinary research can be complex, but it is also of great value, because the merger of basic and clinical skills enhances the pace of developing better treatment options for all cancer patients.

Introducing More Potent PARP Inhibitors

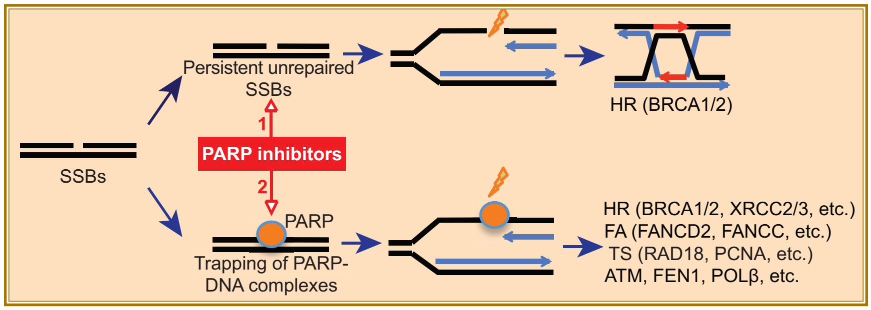
Scientists from CCR have made a discovery about how small molecules that target poly (ADP-ribose) polymerase–called PARP inhibitors–can stop cancerous growth. The findings, reported in *Cancer Research*, may allow clinicians to add more potent PARP inhibitors during treatment, according to the study's lead author, Yves Pommier, M.D., Ph.D., Chief of CCR's Laboratory of Molecular Pharmacology.

When a normal cell's DNA is damaged or mutated, several mechanisms and pathways can come into play to detect and repair the alterations. If the DNA is successfully repaired, the cell survives. If the DNA cannot be repaired, the cell undergoes a form of cellular suicide called apoptosis.

A protein that repairs damaged DNA is poly (ADP-ribose) polymerase, or PARP. When a strand of DNA is broken, or nicked, PARP moves to the damaged site and becomes activated.

Until the current study, PARP inhibitors were assumed to kill cancer cells by a process known as catalytic inhibition. These small molecule inhibitors prevent PARP from building its PAR polymer, a large, branched molecular bandage that wraps around damaged DNA and coordinates with nearby repair enzymes. PARP makes the polymer out of nicotinamide adenine dinucleotide (NAD+) building blocks, which bind to its catalytic site. Because PARP inhibitors also bind to that site, they block NAD+'s access. So the PAR polymer does not assemble, and DNA damage is not removed.

Preclinical studies of PARP inhibitors drew considerable interest because they induced apoptosis in



(Image: Y. Pommier, CCR)

Dual cytotoxic mechanisms of PARP inhibitors. 1: Catalytic inhibition (upper pathway) interferes with the repair of DNA single-strand breaks (SSBs), leading to replication fork damage that requires homologous recombination (HR) repair. 2: Trapping of PARP-DNA complexes also leads to replication fork damage but utilizes additional repair pathways including Fanconi pathway (FA), template switching (TS), ATM, FEN1 (replicative flap endonuclease) and polymerase β.

BRCA-mutated breast tumor cells. These cells already have defective DNA repair, so it seemed that PARP inhibitors blocked an alternative repair pathway that *BRCA*-mutated cancer cells use to fix damaged DNA. Unable to harness that pathway, which is known as base excision repair (BER), breast cancer cells accumulated DNA damage and died. Consequently, PARP inhibitors moved on to clinical trials.

Catalytic inhibition is not the only way that PARP inhibitors slow cancerous growth, however. Pommier and his colleagues have now demonstrated that some PARP inhibitors also trap PARP on DNA by way of a poisonous "allosteric" effect.

"Our data show that when some PARP inhibitors bind to the NAD+ pocket, they tighten PARP binding to DNA," Pommier explains, "so their toxicity may be due to the poisonous complex that forms and prevents replication and transcription." He adds, "The PARP-DNA complex may also have more anticancer activity than catalytic inhibition."

Not all PARP inhibitors have this trapping ability. In fact, PARP-DNA complex formation depends heavily on the chemical structure of the PARP inhibitor. Of the three drugs tested by the CCR research team, in collaboration with James Doroshow, M.D., Deputy Director for Clinical and Translational Research at NCI, only olaparib and niraparib were capable of both catalytic inhibition and PARP poisoning. Another drug, velaparib, was primarily a catalytic inhibitor.

According to Pommier, this helps to explain why velaparib is less cytotoxic to cancer cells than the other two drugs, despite having a similar ability to inhibit PARP's catalytic activity. It suggests that while olaparib and niraparib may be powerful enough for use as single-agent monotherapy, velaparib may be better suited to combination treatments.

Pommier emphasizes that while PARP inhibitors have been developed primarily for *BRCA1*- and *BRCA2*-mutant cancers, they may also be clinically useful in cancers associated with other types of DNA repair deficiency.

To learn more about Dr. Pommier's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?name=pommier.

New Agents for Burkitt's Lymphoma May Reduce Immunosuppression

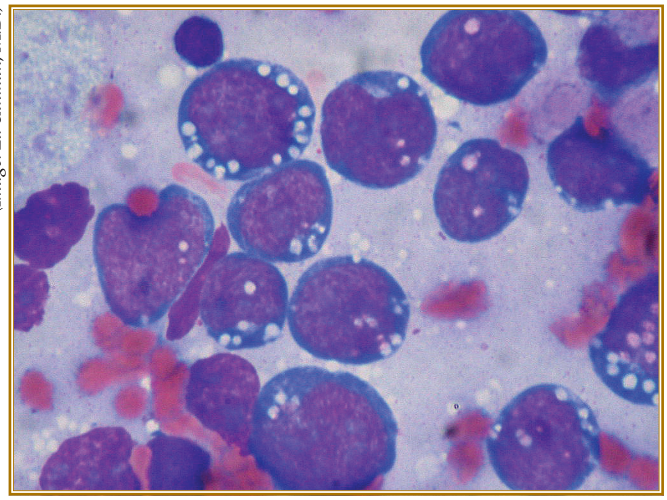
Rare in the United States, but more common in the developing world, Burkitt's lymphoma (BL) is an aggressive form of non-Hodgkin's lymphoma that typically responds well to treatment. The most successful treatments are highly immunosuppressive, however, so doctors usually prescribe them only for patients whom they can monitor and treat for infectious complications, such as the elderly and children in developing countries.

Now scientists have uncovered genetic signatures for BL that show, for the first time, that the illness is molecularly distinct from other lymphomas. Mutated genes and pathways within those signatures might be targets for new, less immunosuppressive therapies, reports Louis M. Staudt, M.D., Ph.D., Deputy Chief of CCR's Metabolism Branch, who led the study. These findings, published in *Nature* last October, may lead to effective, better tolerated treatment therapies for BL.

Staudt's research team showed previously that BL differs genetically from another non-Hodgkin's lymphoma known as diffuse large-B-cell lymphoma (DLBCL). BL has three recognized subtypes. These include a sporadic subtype diagnosed most often in children from developed countries, an Epstein-Barr virus-associated subtype that is endemic in Africa, and an HIV-associated subtype. Furthermore, the team was aware that c-myc—a tumor-promoting oncogene—is always active in this cancer, though the regulatory pathways that cooperate with c-myc were unknown.

Undaunted, the Staudt team set out to identify the specific genes and pathways in BL cells that enable proliferation and survival. They screened biopsy samples from 28 patients with BL and 13 BL cell lines. They then compared the results to sequencing data from DLBCL biopsies and found a striking set of new mutations that were not present in DLBCL, nor in other types of cancer.

(Image: Ed Uthman, M.D.)



Burkitt's lymphoma cells

Among them, mutations affecting the transcription factor TCF3 and its negative regulator ID3 were observed most often, detected in up to 70 percent of the samples from sporadic cases and 40 percent of those from endemic cases.

Staudt's team found that when mutated, TCF3 and ID3 boost the expression of genes and proteins that drive cancer progression. One such protein is the B cell receptor, which detects foreign pathogens during an immune response, and promotes cancer cell survival by activating the PI(3) kinase pathway. The team also tested some of the new PI(3) kinase inhibitors in BL cell lines. "We found that they were toxic to every cell line that we had," Staudt reported. PI(3) kinase inhibitors are far less immuno-suppressive than the chemotherapies currently used in BL treatment.

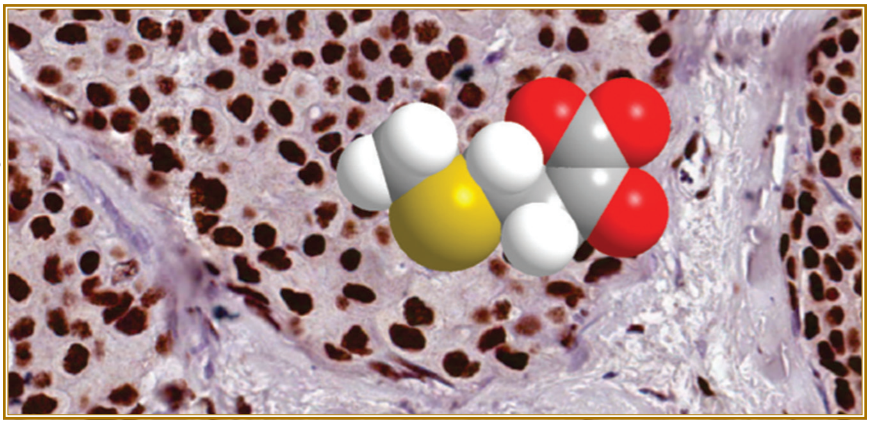
A different set of mutations was found to affect yet another gene controlled by TCF3, known as *CCND3*. This gene encodes for the protein cyclin D3, which regulates key phases of the cell cycle. But when mutated, cyclin D3 drives explosive rates of BL cell proliferation. "So driven by aberrations in the PI(3) kinase and cyclin D3 pathways, the BL cancer cell survives too long and it proliferates too much," Staudt said. Discovery of cyclin D3's involvement in BL may lead to new treatments.

NCI's recently established Center for Global Health plans to investigate these new therapeutic options for patients in the developing world. "But we are also looking for less toxic therapies for all BL patients," Staudt added.

To learn more about Dr. Staudt's research, please visit his CCR website at http://ccr.cancer.gov/staff/staff.asp?name=staudt.

Newly Identified Mechanism Links Obesity with Breast Cancer

(Image: K. Gardner, CCR)



CtBP expression in breast cancer with model of a molecule that can inhibit CtBP activity

Mounting evidence points to obesity as a risk factor for a variety of cancers. Now a new study by CCR scientists shows that excessive carbohydrate metabolism activates a protein that suppresses *BRCA1* and many other genes involved in DNA repair. The protein, called c-terminal binding protein (CtBP), links breast cancer with obesity, diabetes, and other conditions associated with metabolic imbalance, according to a study led by Kevin Gardner, M.D., Ph.D., a Senior Investigator in the Genetics Branch. The findings appeared recently in *Nature Communications*.

Dividing cells rely on the energy provided by carbohydrate metabolism. Through carbohydrate metabolism, the energy contained in sugars such as glucose gets transferred to a high energy intermediate known as NADH, which then gets converted into a substance called ATP that fuels cellular activity.

In previous research, Gardner and colleagues had revealed that NADH, CtBP, and *BRCA1* work in interconnected ways. Specifically, NADH activates CtBP, which itself represses *BRCA1* activity. During carbohydrate metabolism, normal cells produce limited amounts of NADH, while cancer cells generate excess NADH. This results in constant CtBP activation, *BRCA1* repression, and limited DNA repair.

Based on those findings, Gardner speculated that high glucose levels encountered in obesity and diabetes also fuel excess NADH generation and boost cancer risk. "We projected that this could be the link between excessive weight gain and breast cancer," Gardner said. During the study, Gardner also investigated if CtBP regulates other DNA repair genes in addition to *BRCA1*.

To investigate, Gardner's research team used chromatin immunoprecipitation combined with DNA sequencing (ChIP-seq) to determine how many genes CtBP interacts with in breast cancer cell lines. That research provided evidence that CtBP functions as a master regulator for a suite of DNA repair genes. "We identified more than 1,800 gene targets for CtBP, many of which are involved in DNA repair and some of which are linked to hereditary breast cancer," Gardner said. Then the team silenced CtBP with RNA interference and found that *BRCA1*'s expression—and also that of CtBP's other targets—rose in response, resulting in more efficient DNA repair.

Gardner's research also showed that elevated glucose levels—similar to those detected in the cells of diabetic patients—result in higher CtBP activity compared to low glucose levels. Cells exposed to high glucose levels were also less able to repair DNA, but Gardner's team showed it was possible to reverse this effect with small molecules that inhibit CtBP activation.

Then the researchers studied CtBP levels in tumor samples obtained from breast cancer patients. The results showed that differential expression of CtBP-targeted genes predicts poor clinical outcomes, while high CtBP levels in patient tumors predict shorter median survival. "Our data suggests that losing weight improves DNA repair and genome stability in patients who have cancer," Gardner added. "Similarly, obesity activates CtBP and may contribute to more aggressive malignancies."

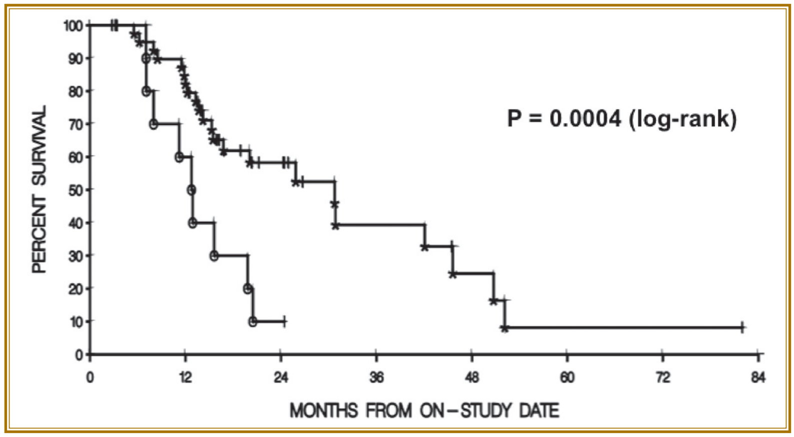
The Gardner team plans to investigate CtBP's role in a variety of other cancers. An additional goal is to identify new drugs that target CtBP and limit its tumor-promoting effects in obese patients.

For more information about Dr. Gardner's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?name=gardner.

Genomic Marker Better Informs Treatment Choices for CRPC

Docetaxel remains the frontline standard of care for castration-resistant prostate cancer (CRPC), which grows without stimulation from male hormones. However, patient responses to this drug are highly variable. Researchers at CCR can now search a patient's genome for a specific genomic variant (called a polymorphism) that predicts lesser responses to docetaxel among CRPC patients.

Led by William Douglas Figg, Sr., Pharm.D., a Senior Investigator in CCR's Medical Oncology Branch, and Staff Scientist, Tristan Sissung, Ph.D., from the Pharmacogenetics Core of the Clinical Pharmacology Program, the clinical team studied a commonly inherited polymorphism in the *cytochrome P450 1B1 (CYP1B1)* gene. Specifically, the 432ValVal polymorphism in *CYP1B 9* (called the *CYP1B1*3* polymorphism) was shown to reduce a patient's survival following docetaxel treatment by more than half—from 30.6 to 12.8 months in combination trials, and from 15.3 to 7.5 months in trials that compared docetaxel alone to prednisone alone. Figg and colleagues conclude that testing for *CYP1B1*3* should guide docetaxel treatment decisions in patients with CRPC, because it could spare many from taking a drug unlikely to help them. Similarly, *CYP1B1*3* testing could inform treatment decisions involving docetaxel and other therapies in breast, ovarian, and non-small cell lung cancers.



(Image: T. Sissung, CCR)

The *CYP1B1*3* genotype is a potential marker for poor prognosis for men with castration-resistant prostate cancer who received docetaxel-based therapy. Men carrying two copies of *CYP1B1*3* (o) had reduced survival times compared to patients carrying at least one copy of the wild-type gene (*).

While examining the mechanism of action for docetaxel, which inhibits microtubule disassembly, Figg and his team noted that this drug was being metabolized similarly by cells whether or not they carried the *CYP1B1*3* variant, based upon clearance data, which was the same for the differing genotypes. They wanted to know what was occurring at the biochemical level. They knew that the CYP1B1 enzyme metabolizes endogenous steroids, including estrogen, so they looked at how estrogen metabolites interact with tubulin, which makes up microtubules. They found that the estrogen metabolite estradiol-3,4 quinone interferes with docetaxel's ability to promote tubulin formation and binds directly with docetaxel, creating a drug-estrogen adduct.

Based on these findings, Figg and colleagues proposed that *CYP1B1*3* interferes with docetaxel therapy by boosting the production of a metabolite that displaces docetaxel from its target and by creating adducts with more limited potency than the drug itself. "Patients who harbor the variant make more estradiol-3,4 quinone, which may work against docetaxel efficacy, while patients who have the wild-type gene make less of it and respond better to the drug, " explains Sissung.

The frequency varies among racial and ethnic groups worldwide, with approximately 20 percent of the Caucasian population harboring the *CYP1B1*3* variant. "We want to limit the number of people who receive docetaxel without experiencing benefits from the treatment," said Figg.

Figg has now patented the use of *CYP1B1*3* genotyping in blood samples to mark patients unlikely to benefit from docetaxel treatment in CRPC. "We think this genetic marker has value, and we are willing to work with other groups to validate the findings prospectively," he said. "The goal is to make sure this test reaches the market so it can be used to improve treatment planning."

The technology is available for licensing through the NIH Office of Technology Transfer. In addition to licensing, it is also available for collaborative research opportunities with Figg.

To inquire about licensing the technology, please contact Sabarni Chatterjee, Ph.D., at chatterjeesa@mail.nih.gov.

To learn more about Dr. Figg's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?name=figg.

Recent CCR Awards

Keio Medical Science Prize
Keio University, Japan

For outstanding contribution to the field of medicine or life sciences

Steven A. Rosenberg, M.D., Ph.D.
Chief, Surgery Branch

2012 Lifetime Achievement Award for Scientific Contributions
Institute of Human Virology at the University of Maryland School of Medicine

For lifetime achievement in science and medicine

Thomas Waldmann, M.D.
Chief, Metabolism Branch

Aultman Cancer Center's Cancer Research Award
For lifetime commitment to advancing cancer research

Marston Linehan, M.D.
Chief, Urologic Oncology Branch

2012 Aaron B. Lerner/PASPCR Special Lecture
PanAmerican Society for Pigment Cell Research

For his contribution as an outstanding researcher making a significant impact on the field of pigment cell research

Glenn Merlino, Ph.D.
Chief, Laboratory of Cancer Biology and Genetics

Tom Connors Lecturer Award
British Association for Cancer Research

Lee Helman, M.D.
Scientific Director for Clinical Research, CCR

2013 Ruth Kirschstein Diversity in Science Award
The American Society for Biochemistry and Molecular Biology

For an outstanding scientist who has shown a strong commitment to the encouragement of under-represented minorities to enter the scientific enterprise and/or to the effective mentorship of those within it

Peter Blumberg, Ph.D.
Laboratory of Cancer Biology and Genetics

Gregory T. O'Conor Award for Outstanding Contributions to Haematopathology
International Network for Cancer Treatment and Research

Elaine Jaffe, M.D.
Laboratory of Pathology

Joanne Vandenberg Hill Award
M.D. Anderson Cancer Center

For excellence in pathology

Maria Merino, M.D.
Laboratory of Pathology

Bridge 2012 Award
Melanoma Foundation Onlus

For outstanding and lifelong contributions to cancer research

Giorgio Trinchieri, M.D.
Chief, Laboratory of Experimental Immunology

Elected to Fellowship in the American Academy of Microbiology
Dennis Klinman M.D., Ph.D.
Laboratory of Experimental Immunology

2012 American Association for the Advancement of Science Fellows
For distinguished contributions to cancer prevention research, particularly for the discovery and validation of molecular targets and for dietary interventions to prevent cancer

Nancy Colburn, Ph.D.
Chief, Laboratory of Cancer Prevention

For outstanding contributions to the field of lymphocyte signal transduction, including pioneering studies integrating cellular and molecular imaging with genetic, biophysical and biochemical research approaches

Lawrence Samelson, M.D.
Chief, Laboratory of Cellular and Molecular Biology

Recently Tenured CCR Scientists

Chand Khanna, D.V.M., Ph.D.
Pediatric Oncology Branch

William Douglas Figg, Sr., Pharm.D.
Medical Oncology Branch

Jeffery Gildersleeve, Ph.D.
Chemical Biology Laboratory

Staff News at CCR

Announcements

(Photo: R. Baer)

Electron Kebebew, M.D.

Electron Kebebew has been named Chief of CCR's newly established Endocrine Oncology Branch. He completed his medical degree, surgical residency, and an NCI surgical oncology basic science fellowship at the University of California, San Francisco (UCSF). He then became a member of the UCSF surgical faculty and the UCSF Helen Diller Family Comprehensive Cancer Center. Kebebew joined CCR's Surgery Branch in 2009 as the Head of the Endocrine Oncology Section. He is an internationally recognized endocrine surgeon. His research focuses on elucidating the molecular mechanism in endocrine cancer initiation and progression, with the goal of identifying therapeutic targets and diagnostic and prognostic markers for endocrine tumors.

New Tenure-Track Scientists

(Photo: B.Branson)

Alexander Kelly, Ph.D.

Alexander Kelly joins CCR's Laboratory of Biochemistry and Molecular Biology. His research seeks to elucidate the fundamental molecular and biophysical mechanisms that ensure the equal distribution of genetic information during cell division, with a focus on multi-protein complexes that govern chromosome structure and function. His lab will investigate how dysregulation of these processes can lead to aneuploidy and genomic instability, both of which are hallmarks of cancer cells.

(Photo: B.Branson)

Frank Maldarelli, M.D., Ph.D.

Frank Maldarelli is now a tenure-track investigator in CCR's Host-Virus Interaction Branch, the clinical arm of the HIV Drug Resistance Program. His research focuses on elucidating mechanisms underlying the emergence of viral resistance in vivo, the dynamics of infection under treatment, and the role of resistance mutations in the efficacy and failure of subsequent treatments.

(Photo: R. Frederickson, SPGM, FNL)

Jordan Meier, Ph.D.

Jordan Meier joins CCR's Chemical Biology Laboratory. His research focuses on synthetic molecular approaches to study and modulate cofactor-mediated signaling pathways at the interface of cancer cell metabolism and gene expression.

(Photo: B.Branson)

Stavroula (Voula) Mili, Ph.D.

Voula Mili joins CCR's Laboratory of Cellular and Molecular Biology. Her research focuses on understanding the mechanisms and regulation of RNA localization in mammalian cells; the effect of localized translation on protein function; and the contribution of these processes in disease.

(Photo: B.Branson)

Martin Schnermann, Ph.D.

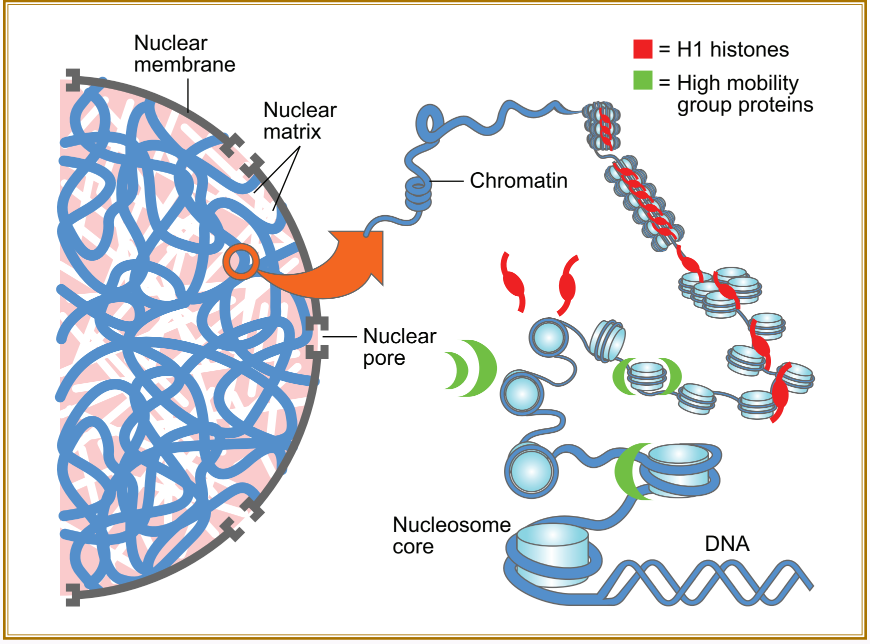
Martin Schnermann joins the CCR's Chemical Biology Laboratory. His research focuses on organic synthesis and its application to uncovering the mechanisms of action of bioactive small molecules and to developing new classes of potential therapeutic agents.

(Photo: B.Branson)

Kandice Tanner, Ph.D.

Kandice Tanner joins CCR's Laboratory of Cell Biology. Her research focuses on the physical mechanisms of morphogenesis from single cell to tissues in 3D cell cultures and in vivo in animal models.

CECB: Capturing Dynamic Changes in Chromatin



Deoxyribonucleic acid (DNA) contains the instructions a cell uses to build proteins essential to life. The complete supply of DNA is packaged inside the cell as macromolecules called chromosomes; the complete set of chromosomes is called a genome. Each chromosome houses many working units called genes, and each gene sits within tightly coiled DNA strands that are wrapped around eight histone proteins in a package called a nucleosome. Chromatin is the full collection of these nucleosomes. The genome is stored in the cell nucleus.

Trends in research sometimes veer away from the path of important discoveries, and the study of chromatin's architecture is a good example of this. Until recently, scientists were not convinced that nucleosomes or histones interact dynamically with DNA. DNA topology was viewed statically, as if its primary sequences alone determined its actions. Many scientists dismissed the idea that DNA topology plays a regulatory role in chromosome biology.

Today scientific perceptions of chromatin have shifted radically from this static view to a dynamic model, in part, because of the outstanding research conducted by NCI's Center of Excellence in Chromosome Biology (CECB), one of five Centers of Excellence within NCI's Intramural Research Program. CCR Director Robert Wiltrout, Ph.D., created the collaborative infrastructure in 2006 to unite diverse research skills and to investigate the multiple factors that enable dynamic changes to occur in DNA topology. Currently, over 40 NCI investigators are members or serve on a steering committee, chaired by Gordon Hager, Ph.D., Chief of CCR's Laboratory of Receptor Biology and Gene Expression, that guides CECB activities.

Working through the Center, CCR scientists peer into the nucleus and study how cells regulate rapid responses to physiological stimuli in real time. With this new approach, CECB scientists have redefined chromatin's role in the cell.

"Chromatin and chromosome biology is only in its infancy," explains Hager, "yet epigenetics has already become central to a mechanistic understanding of nuclear function."

CECB investigators have shown that DNA topology changes frequently and that those changes have consequences. They have redefined the operational mechanisms by which proteins and DNA interact during replication, RNA transcription, and DNA repair.

The CECB's success continues as its members share their prolific findings with colleagues across the NIH campus and with the extramural community. The *NCI Symposium on Chromosome Biology*, hosted every 18 months by CECB, has grown into an international conference. The most recent symposium, "Epigenetics in Development," was held in April on the NIH campus in Bethesda, Md., and attracted about 700 attendees from around the world.

The CECB also hosts a quarterly trans-NIH workshop with the NIH Chromatin Interest Group. The goal of this workshop is to cultivate interactions among major laboratories in chromosome biology, and to enhance the education and development of junior investigators and fellows in CECB laboratories. Postdoctoral fellows working in chromosome biology have also created and sponsor their own seminar series, called Chromatin Decode. This series provides a more informal forum for postdoctoral fellows to present their ongoing work.

The increasing interest in CECB, which extends beyond NIH, can be largely attributed to the singular

Center of Excellence in Chromosome Biology

discoveries made by its members. Early in the Center's young life, CECB member Tom Misteli, Ph.D., reported insightful results after visualizing a set of mouse chromosomes and analyzing their positions in the 3D space of the cell's nucleus. Later he and his team did similar work in human cancer cells. Their evidence pointed to the possibility that the arrangements and spatial relationships among chromosomes are far from random. Misteli and his research team demonstrated further that chromosomes actually cluster into distinct topological neighborhoods, and that the resulting positional patterns differ depending on cell type. They discovered that these patterns are not just passive bystanders in genome function. Quite the contrary, they play a key role in chromosome rearrangements. Using cancer cells, the Misteli team showed that proximity, in part, dictates the nature of genomic rearrangements.

Working in this same area of research, the Misteli lab recently also reported that broken chromosomes—that do not reanneal—are unable to undergo dynamic motion within the nucleus. This surprising result is now thought to be an important cancer suppressing mechanism.

Chromosome biology researchers also tackle another area of dynamic chromatin, namely, the dynamic binding of transcription factors, using steroid receptor proteins as an example. Hager and colleagues discovered that a large fraction of genomic receptor binding requires a localized open conformation of the chromatin prior to hormone signaling. They demonstrated that cell-specific-proteins determine tissue selective hormone-driven transcription by opening chromatin at subsets of all genomic receptor binding elements. For mammary cells, a constitutive nuclear protein called AP1 maintains chromatin in an open state at these elements, keeping them ready for action. Importantly, the CCR scientists showed that baseline chromatin accessibility is actively maintained by constitutive AP1 binding, keeping it ready for more specific actions. It directs traffic as inducible transcription factors arrive in response to hormone signaling.

CECB member Mirit Aladjem, Ph.D., realized that a proper start of DNA replication may require proteins that bridge distant chromosomal sequences together. This fact did not intimidate Aladjem from tackling the mechanisms involved and from publishing the first comprehensive mapping of the locations of all replication starting points in several cancer genomes. She realizes that unraveling the chromatin dynamics during replication will be very important for understanding the regulation of cell growth, and that sequences that affect replication might improve the future design of effective gene therapy vectors. (See "It Starts with a Choice," page 22.)

In addition to studying topology, CECB scientists also look carefully at post-translational modifications to chromatin, an area of research called epigenetics. While studying the role of epigenetics in altering chromosomal architecture, CECB member Charles Vinson, Ph.D., showed how methylation of CpG sites within chromatin actually recruits sequence-specific transcription factors that are essential for some tissue-specific gene expression.

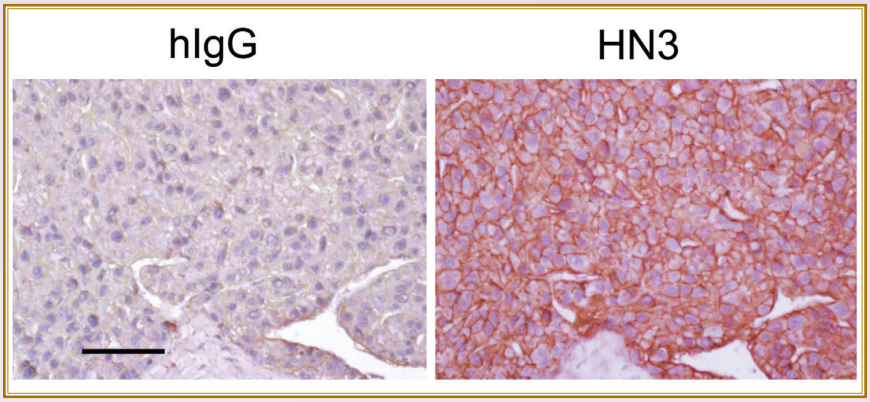
Aware that DNA in B cells routinely breaks and recombines genetic information to build proteins with specific antibody shapes, CECB member Andre Nussenzweig, Ph.D., realized that this function in itself could increase chromosomal translocations in B cell lymphomas. So he and his research team pursued the abnormal chromatin interactions that occur in B cell lymphoma and discovered how normal B cells protect themselves against accumulating the excessive DNA breaks that can lead to unwanted translocations.

These and similar discoveries keep coming from the laboratories of CECB members. The Center's prolific work in support of the importance of chromatin's dynamic architecture has resulted in a special issue of the journal *Biochimica et Biophysica Acta (BBA) Gene Regulatory Mechanisms* entitled "Chromatin in time and space." Elsevier released the print version, containing 31 articles authored by CECB and NIH chromatin group scientists, in July 2012.

Paying attention to chromosomal topology is a new trend that has changed the field of chromosome biology. By characterizing chromatin's three-dimensional organization and dynamics, members of CCR's CECB are enlightening the scientific community about how chromatin's topology exerts regulation on gene activities and how chromatin aberrations lead to disease.

To learn more about the Center of Excellence in Chromosome Biology, please visit its Web site at http://chromosomebiology.nci.nih.gov.

Novel Antibody Targets Glypican-3 in Liver Cancer



(Image: M. Ho, CCR)

CCR scientists generated a new human single-domain antibody, HN3, that recognizes GPC3 for liver cancer therapy. The human antibody strongly bound HCC cells (right) and inhibited tumor cell proliferation via inactivating Hippo/yap signaling. Furthermore, HN3 significantly inhibited growth of HCC tumor xenografts in mice. hIgG is pooled human IgG used as control. Scale bar, 100 μm.

New treatments for liver cancer are greatly needed, because hepatocellular carcinoma (HCC), the most common type of liver cancer, is particularly insensitive to chemotherapy. Surgery is standard for HCCs caught early, but only a third of cases are identified at this stage. Antibody therapy offers a potential alternative for later-stage tumors.

An attractive antibody target is glypican-3 (GPC3), a cell surface associated-protein that is overexpressed on HCC cells but not on normal liver tissue. While its exact function is not well understood, GPC3 seems to help regulate cell growth. Mitchell Ho, Ph.D., in CCR's Laboratory of Molecular Biology, and his colleagues decided to investigate the role of GPC3 in HCC and develop GPC3-specific antibodies. Their findings were recently published in the *Proceedings of the National Academy of Science*.

To identify antibody domains targeting GPC3, the investigators screened a phage library of heavy chain variable domains, which can interact with small pockets or other regions that full-length antibodies cannot, for their GPC3-binding ability. The library was previously described by Dimiter Dimitrov, Ph.D., in CCR's Nanobiology Program. After four rounds of phage panning, Mingqian Feng, Ph.D., a Postdoctoral Fellow in the Ho lab, identified four interacting domains. The HN3 domain had the strongest association and was used for subsequent studies.

The researchers cloned the HN3 domain into a vector that fused it with the constant region of a human antibody. Only cells that expressed GPC3 bound to the antibody. To see what effect HN3 had on cell growth, the researchers treated four HCC lines with the antibody. HN3 significantly reduced growth in three of the four lines at 0.1μM and reduced the growth of the fourth at 1.0μM. A cell line that normally lacks GPC3 was not affected by HN3.

To understand how HN3 caused growth arrest, the scientists examined several pro-growth signaling pathways. HN3 treatment decreased phospho-Erk and phospho-Akt levels in four HCC lines. Levels of phospho-yap, an inactive form of this Hippo pathway member, increased in three of the lines. Expression of cyclin D1, a yap target, decreased in all four. Because Erk and Akt can affect Hippo signaling, these results suggested that yap facilitates HN3-mediated cell cycle arrest.

To test this idea, the researchers over-expressed constitutively-active yap or knocked down yap in an HCC line and evaluated HN3-induced growth arrest. Cells over-expressing active yap proliferated more and were insensitive to HN3. Conversely, cells lacking yap had much lower growth rates. Adding HN3 antibody had no effect. These studies indicate that yap is an important target of HN3 and GPC3 signaling.

Finally, the researchers treated mice bearing HCC tumors with HN3 or a control twice a week. HN3 treatment significantly reduced the size of tumors from two HCC lines. The scientists also detected increases in phospho-yap and decreases in cyclin D1 and phospho-Erk in treated tumors. The investigators noted no antibody-related toxicities and suggested that HN3 should undergo further testing as a potential therapeutic for liver cancer.

To learn more about Dr. Ho's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?name=ho.

In Conversation: Research Fellow Junfang Ji, Ph.D.

(Photo: R. Baer)

Junfang Ji, Ph.D.

CCR: Junfang, you work on hepatocellular carcinoma, which is a particularly aggressive cancer, especially in males. What prompted you to take a translational research approach to this cancer?

Junfang: I started working on liver cancer after coming to CCR to work with Xin Wang, Ph.D., in the Laboratory of Human Carcinogenesis. As a Ph.D. student at Peking University Medical College, in Beijing, my work focused on oncogenic function and DNA repair. From there, I kept thinking whether and how our scientific findings could be connected to patient benefits. Translational research is the conduit. It is highly challenging but also rewarding. CCR is a great environment for doing translational research—the questions I must address are more complicated, but there are lots of resources here and opportunities for collaboration.

CCR: What are the overall goals of your investigations here?

Junfang: Liver cancer has tremendous heterogeneity from patient to patient and also within single tumors. In the big picture, we try to understand the genetics of tumor heterogeneity so we can stratify patients by their gene profiles. We also aim to identify key driver genes for each cancer subgroup. Our hope is that by targeting driver genes, we can increase the survival of patients with liver cancer. This would allow us to maximize treatment efficiency.

CCR: What are you learning about intertumor heterogeneity, or the way tumors differ among individual patients?

Junfang: Males and females have very different liver tumor biology: Females are less likely to develop liver cancer, and when they do, they have better survival. We found that compared to males, females express much higher levels of a microRNA called miR-26, which acts as a tumor suppressor. We also find that miR-26 is reduced in tumor versus nontumor tissues, and that many immune-associated pathways are activated in tumors with low miR-26 expression.

CCR: Do you see translational opportunities for miR-26 in the clinic?

Junfang: Yes. We found that patients with low levels of miR-26 respond to immunomodulating therapy with interferon alpha, while patients with high levels do not. Now we are trying to make a diagnostic—the miR-26 DX test—for use in choosing patients for interferon-α treatment. This could allow for more efficient use of treatment resources.

CCR: What are you learning about intratumor heterogeneity?

Junfang: Some cancer cells in the liver tend to be very hard to treat. Even after surgery and/or chemotherapy they form new tumors—these are the hepatic cancer stem cells (HepCSC). We isolated HepCSCs using the EpCAM surface marker and found that they have an aggressive phenotype when implanted into immunocompromised mice. We also found that EpCAM-positive HepCSCs highly express a microRNA called miR-181.

CCR: Does that make miR-181 a potential drug target in hepatic cancer stem cells?

Junfang: This is something we are investigating. One problem is that miR-181 is also highly expressed in normal hepatic stem cells. So if we silence it in tumors, we also likely suppress it in these other cells and that is a side effect that we do not want. But recently we discovered another group of microRNAs that seem to be very specifically expressed in hepatic cancer stem cells and we are happy to see that. These data will be published soon.

CCR: What do you hope to do after leaving CCR?

Junfang: I am looking for a faculty position and would like to continue my work on sex-related differences in cancer incidence and mortality. The underlying molecular mechanisms in sex-related carcinogenesis are poorly understood, but our data with microRNAs might be providing important clues. I would like to work on decoding the biological differences between male and female and liver cancers at multiple levels with an overarching goal of developing tools for early diagnosis, prognosis, therapeutic stratification, and effective therapy.

Virtual Entity
Yields Real-Time Results

Genomic technologies have revolutionized cancer research, but not without analytical challenges. No single researcher can manage the vast datasets generated by next-generation sequencing and other modern genomic tools. Harnessing those tools in the pursuit of translational advances increasingly compels team science and multidisciplinary collaboration. Toward that end, NCI's Center of Excellence in Integrative Cancer Biology and Genomics (CEICBG) was created in 2008 to unite experts in cancer biology with colleagues in bioinformatics. Working collaboratively, active participants drawn from across NCI use genomics technology to advance basic science discoveries and clinical research applications for the prevention, diagnosis, and treatment of cancer.

Sponsored by CCR, CEICBG is a virtual entity. It exists through the interactions of its members, who are organized into five focus areas (called Subcommittees): Biomarkers and Molecular Targets, Genomics Approaches, Human Genomics and Genetics, Cancer Inflammation, and Integrative/Systems Biology and Bioinformatics. Participating scientists meet regularly to identify potential collaborations within and outside the CEICBG. The group hosts an annual one-day meeting offering enriching lectures and it also hosts a biennial symposium on translational research. The first symposium covered translational genomics, while the second, convened last year, presented clinical applications for next-generation sequencing. It drew more than 1,000 attendees from around the world. The Center plans to host their next symposium in 2014.

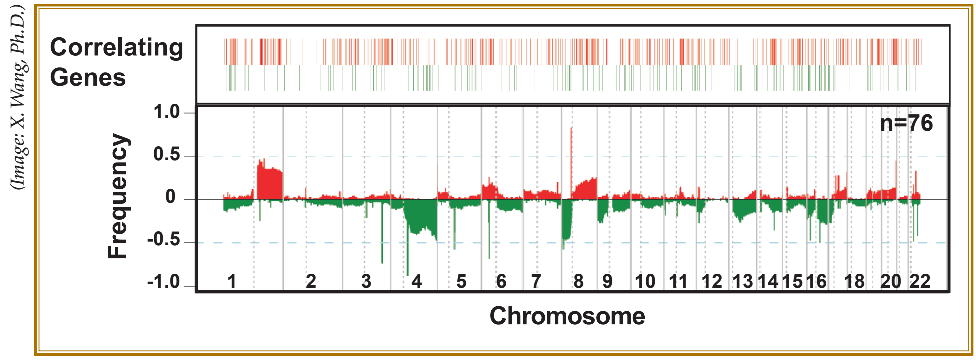
"In CEICBG, we investigate cancer in the broadest sense," said Snorri Thorgeirsson, M.D., Ph.D., Chief of CCR's Laboratory of Experimental Carcinogenesis, and Head of the CEICBG, "from tumor biology, to gene expression, to whole genome sequencing, all the way to potential therapies."

(Photo: R. Baer)

Snorri Thorgerisson, M.D., Ph.D., and Patricia Johnson in CCR's DNA Sequencing Core

Collaborations Enable Insights

CEICBG was deliberately structured to unite scientists with complementary research focus areas. Xin Wang, Ph.D., Deputy Chief of CCR's Laboratory of Human Carcinogenesis (LHC), and Thomas Ried, M.D., a Senior Investigator in CCR's Genetics Branch, both members of the Biomarkers and Molecular Targets Subcommittee, collaborate on research projects to develop and validate biomarkers in cancer treatment that offer an



In the somatic cells from 76 clinical specimens, significant differences in gene copy numbers were noted at specific locations in the genome. Frequencies of samples showing copy number increases at a particular site are shown in red, and those showing copy number decreases are shown in green.

excellent example of CEICBG's fruitfulness.

Wang explains how he and Ried study genomic data—which is generated in his laboratory—to segregate tumors into discreet clusters of biomarkers that may eventually help clinicians match the right drugs with the right patients. Biomarker research may one day differentiate "driver" mutations that promote tumor growth from "passenger mutations" that do not. "If we can identify druggable tumor drivers, we can eliminate tumors completely," Wang said.

For help with his data analysis, Wang turns to Paul Meltzer, M.D., Ph.D., Chief of CCR's Genetics Branch and a member of the Genomics Approaches Subcommittee. Throughout the CEICBG, Meltzer shares new analytic technologies with his colleagues. Recently, he and Wang pinpointed driver mutations in liver cancer by looking at changes in a parameter called somatic copy number alteration. As Meltzer explains, normal cells ordinarily have two copies of each gene, one inherited from each parent. But because tumors are genomically unstable, copy numbers can vary in cancer—tumor suppressor genes may be deleted, for example, while genes that drive cancer progression may be amplified, so hundreds of copies may be present.

In their collaboration—published last year in *Gastroenterology*—Wang and Meltzer measured copy number changes to find driver mutations involving 10 genes on chromosome 8p, which predict poor outcomes. They also concluded that the expression pattern of these driver genes could be useful in tumor diagnosis and staging.

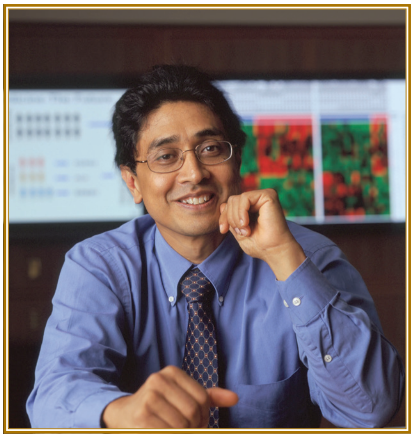
Wang's collaborations with CEICBG members and researchers in China have already produced new diagnostics for liver cancer that are now being evaluated in clinical trials; one is in China and the other in the U.S. (clinicaltrials.gov: NCT01681446). The first trial relies on assays that predict the response to interferon-alpha therapy based on the expression of microRNA-26, which is a small, non-coding RNA with tumor-suppressor function. Patients who express low levels of this biomarker respond far better to the drug than those with higher levels, Wang's research has shown. "So this is a good example of precision medicine," he said. The trial in the U.S. screens for a specific gene signature that predicts tumor relapse. Described in a 2010 paper in *Cancer Research*, grouping of 161 genes predicts overall and disease-free survival, which is especially useful for patients with early-stage disease. The diagnostic incorporates those findings, Wang said, and is used to select patients for additional and aggressive adjuvant therapy.

> Wang's collaborations with CEICBG members and researchers in China have already produced new diagnostics for liver cancer that are now being evaluated in clinical trials.

The Human Genomics and Genetics focus area fosters collaborations aimed at finding harmful gene variants through genome-wide association studies (GWAS). Curt Harris, M.D., Chief of LHC, says one of his team's ongoing projects reflects the Subcommittee's mission. Together with scientists from across NCI and beyond, Harris and colleagues are conducting the first GWAS study of lung cancer in African Americans. This population has disproportionately higher rates of lung cancer and also has a more aggressive form of the disease.

GWAS studies are often large (the current lung cancer study enrolls roughly 5,000 people), so the team works with collaborators located throughout the U.S. who collect tissue

(Photo: R. Baer)



Javed Khan, M.D.

samples and clinical data. One of their NCI research partners is Stephen Chanock, M.D., in NCI's Division of Cancer Epidemiology and Genetics (DCEG). Chanock's laboratory handles the analytical component of the study—specifically, by looking for genetic variants in lung tissue—while Harris's team handles the integrative molecular epidemiology, which combines molecular genetics with traditional epidemiology. "We are looking for unique variants in African Americans that we can use for diagnostic and prognostic purposes," said Harris. "And that will lead to functional analyses focused on understanding how these variants contribute to tumor risk and progression."

The Integrative/Systems Biology and Bioinformatics focus area applies a range of analytical and bioinformatic approaches to find cancer biomarkers for use in the clinic and in drug development. "We try to integrate high-throughput "omic" data into a coherent story of what is happening in the cancer cell," said Javed Khan, M.D., a Senior Investigator in CCR's Pediatric Oncology Branch (POB). "That tends to involve a lot of high-end computational analysis and integration of large datasets. Ideally, we can identify the genes and pathways that drive a particular cancer, and then that leads to potential treatments."

> We try to integrate high-throughput "omic" data into a coherent story of what is happening in the cancer cell.

Khan's laboratory is now engaged in a new study opening soon that illustrates this approach. His collaborator is Brigitte Widemann, M.D., also a POB Senior Investigator, who works on a rare and aggressive illness known as malignant peripheral nerve sheath tumor (MPNST). This cancer of connective tissues surrounding the nerves often arises in pediatric patients with a germline *NF1* mutation on chromosome 17. All patients with *NF1* mutations develop neurofibromatosis type 1; a condition that produces skin spots, benign and/or cancerous nerve tumors, bone abnormalities, and other symptoms. Roughly 25-40 percent of *NF1* mutant carriers also develop plexiform neurofibromas, which are noncancerous nerve tumors that cause pain and disfigurement. Plexiform neurofibromas can also transition to MPNST, which is often fatal and for which the only available standard treatment is surgery.

Together with Douglas Stewart, M.D., in DCEG, Widemann and Khan hope to find biomarkers that predict the malignant shift from plexiform neurofibroma to MPNST. Widemann's role will be in the clinical evaluation of the patients, and the selection of suspect lesions for biopsy, as well as adjacent tissues that she believes might remain free of the cancer. She will then turn those biopsies, along with blood samples, over to Stewart and Khan. And they, in turn, will sequence the tissues and perform additional bioinformatic analyses aimed at finding predictive biomarkers, and possibly targets for therapy. Widemann's enthusiasm is evident as she describes her vision for the future, "This is optimal sharing of mutually beneficial expertise. We run one of the largest *NF1* clinics in the country, so we can do the expert phenotyping while Khan and Stewart do the expert genomic studies and biological validation."

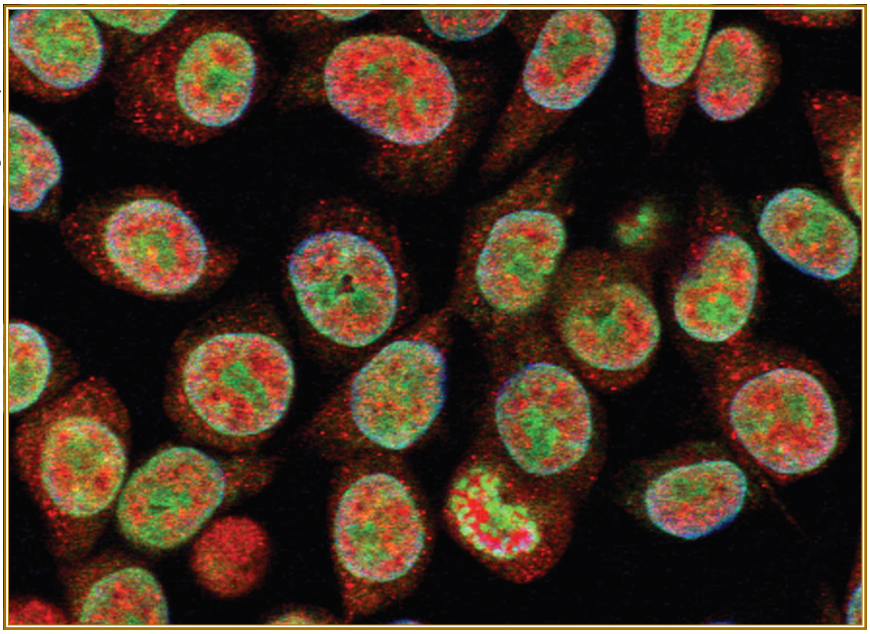
Core Facilities Offer Analytical Support

In addition to fostering collaborations, the CEICBG works at expanding use of the core facilities that NCI researchers rely on for sequencing and analytical support. According to David Goldstein, Ph.D., Head of CCR's Office of Science and Technology Partnerships, the Sequencing Facility run by SAIC, which supplies support and assistance at each phase of the sequencing process, is important in that respect. "This facility has made a significant impact on research at CCR," Goldstein said. The Sequencing Facility offers the latest in massively-parallel technologies, which allow them to generate whole genome analyses in a matter of days.

CCR researchers use the core to study the heterogeneity that exists within and between tumors and to examine many other aspects related to tumor biology: They discover how genomes are rearranged and altered, which regulators are active, and how epigenetic changes affect the cell. And all of this helps them to identify potential drug targets.

But between the sequencing and its therapeutic promise, Goldstein explains, lies a major bottleneck: Enormous and often overwhelming

(Image: S. Garfield, CCR)

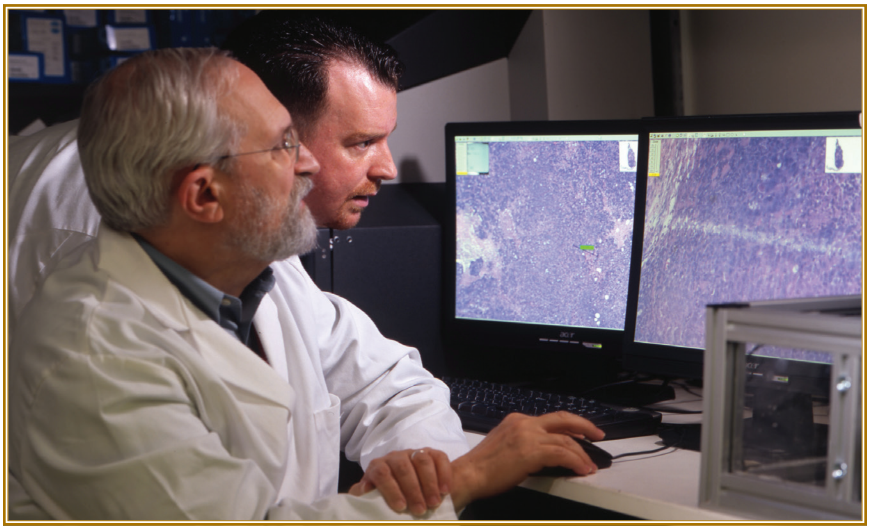


Confocal microscopy image of colon carcinoma.

amounts of data. To address that issue, Goldstein's office helped create the CCR Bioinformatics Core, which was launched in January 2011 to provide analytical support to CCR scientists who do not otherwise have access to bioinformatics. By increasing understanding of bioinformatics techniques and processes among CCR scientists, this Core empowers them to perform basic, informed analyses for their research projects.

Similarly, Meltzer directs the Clinical Molecular Profiling Core, which facilitates the collection of biological data on tumors entered into a CCR clinical trial. Available to scientists throughout NCI, the core offers a range of analytical capabilities: gene expression profiling, comparative genomic hybridization, high-density single-nucleotide polymorphism analysis, and more. The Core spares clinical investigators from having to learn how to use these technologies on their own.

(Photo: R. Baer)



Paul Meltzer, M.D., Ph.D., and colleague

Yet another technology resource, the Confocal Microscopy Core Facility, offers capabilities for tracking live cells and cell components over time—even when in living animals. Confocal microscopy uses lasers to track fluorescently-tagged proteins, cells, and tissues. Available to all NCI staff, the technology's advantage lies in its ability to image specific cells and biomolecules; this is unlike other high-throughput analytical tools that take average measures from cells and tissues mixed together, explains Susan Garfield, M.S., the core's director. Applied to cancer research, scientists use the technology to study protein expression patterns and markers that might change during tumor evolution.

Collaborations Share a Vision

CEICBG's main goal is to use advanced analytic technology to define homogenous clusters of patients, who can then be treated with appropriate therapies. "This is what everyone in the field wants to do," Thorgeirsson said. "So in that sense, CEICBG is a vehicle within CCR and NCI that drives and accelerates translational applications built upon the immense amount of basic research data available here." By bringing expertise in a number of scientific focus areas together to advance cancer research, CEICBG shortens the time between discovery and patient benefit.

For more information about the NCI Center of Excellence in Integrative Cancer Biology and Genomics, please visit its Web site at https://ccrod.cancer.gov/confluence/display/COEICBG/Home.

FEATURE

Training the Next Generation of Cancer Researchers

Staffed by nearly 250 principal investigators (PIs) and linked to the world's largest, publicly funded research hospital, CCR offers a wealth of opportunities for graduate and postgraduate training. At CCR, predoctoral students along with postdoctoral and clinical fellows work at the intersection of basic science and clinical medicine. And with ready access to patients, clinical trials, tumor samples, and advanced technology, CCR's aspiring investigators pursue translational research in a collaborative setting that puts a high priority on professional development.

Students and postdoctoral fellows come to CCR with wide-ranging backgrounds and goals. Some medical doctors (MDs) come for the clinical experience, and the chance to put new treatments to work at the bedside, whereas some PhDs come for translational research experience. Others lean towards more basic research in the laboratory and the promise of developing life-saving therapies for patients with cancer.

(Photo: B. Branson)

Alan Wayne, M.D., Narali Shah, M.D., and Kara Jarnagin, a patient.

Taking the Clinical Track

Nirali Shah, M.D., was drawn to CCR for the clinical research training opportunities. After finishing her combined residency in pediatrics and internal medicine, Shah knew that she still wanted to care for patients, but she also envisioned a career in cancer research. So three years ago, Shah came to CCR—with its stellar record for training physician-scientists—for a fellowship in pediatric hematology/oncology. Administered by CCR's Pediatric Oncology Branch (POB) and The Johns Hopkins University, the fellowship has been, Shah reports, "the right fit for her career goals." It gives her training in oncology, in addition to extensive clinical trial experience.

Guided by her mentor, Alan Wayne, M.D., formerly of POB, Shah focuses on new treatments for pediatric leukemia. And over time, she has worked up to her current role: Serving as a lead associate investigator responsible for interfacing with patients, enrolling them in clinical protocols, administering daily care, and mentoring first-year fellows. Meanwhile, Shah also finished a Masters of Health Science degree in Clinical Research, which is offered by the NIH through Duke University. For her thesis project, she focused on the evaluation of pediatric leukemia patients who relapse after bone marrow transplant.

"I think the resources here are unparalleled," Shah said. "When you start a fellowship, you envision some specific goals that you would like to achieve. For a physician who wants to pursue clinical research,

> CCR leadership supports mentoring not just with words, but with specific initiatives designed to enhance the training experience and smooth transitions towards employment.

no other institution offers as broad an experience in clinical trials and protocols as CCR, making this the best place to train."

Training in Basic Research

While Shah was drawn by CCR's clinical training opportunities, Willie Wilson, Ph.D., was attracted by a postgraduate position that could enable him to develop his basic research skills in cancer biology. As a postdoctoral fellow with Glenn Merlino, Ph.D., a CCR Deputy Director and Chief of the Laboratory of Cancer Biology and Genetics, Wilson investigates surface markers on melanoma cells that might predict their response to treatment. "Our overall aim is to see if the markers can guide precision therapy," he said. For him, training at CCR poses a number of advantages: The core facilities in flow cytometry, histology, sequencing, and microarray analysis are key for his research, and so is his proximity to the NIH Clinical Center, which supplies him with human melanoma samples. Wilson explains, "We work with animal models for melanoma, but we also need to see if the markers they express are found in human specimens."

CCR's translational focus is such that PIs and their trainees interact routinely with clinical-based scientists. "And in that sense, the clinical staff also help with mentoring," Merlino said. For instance, Wilson works with Nicholas Restifo, M.D., a Senior Investigator in CCR's Surgery Branch, on efforts to find surface markers that suppress antitumor T-cell activity.

(Photo: R. Baer)

Glenn Merlino, Ph.D., speaks with Postbacculárate Fellow Pravin Mirsha, Postdoctoral Fellows, Willie Wilson, Ph.D., and Prasun Mishra, Ph.D., and high school student trainee, Azam Husain.

A Commitment to Mentoring

Jonathan Wiest, Ph.D., Director of CCR's Office of Training and Education, emphasizes that students and fellows depend on mentoring as much as they do on CCR's technical and scientific resources. Mentoring is built into the culture at CCR where hundreds of postdoctoral fellows, clinical fellows, postbaccalaureate students, and even high school students train every year. "CCR leadership supports mentoring not just with words, but with specific initiatives designed to enhance the training experience and smooth transitions towards employment," Wiest explains. Some of these initiatives include courses on scientific management, grant-writing workshops, and a peer-based editorial board that offers input on submitted manuscripts. But Wiest also points out that initiatives such as this can only go so far. Mentoring's most important aspect is the communication between PIs and their trainees. "Without productive communication, it is difficult for students and fellows to set long-range goals that go beyond those of the next experiment, " he said.

Merlino feels that a good mentor bears responsibility for the job prospects of the staff scientists, postdoctoral fellows, and graduate students who work with him. "I measure my mentoring success by their success when they leave my laboratory," he said. "That is the litmus test." And effective mentoring, Merlino adds, has both passive and active components. The passive component entails making the laboratory an exciting place to work. Ideally students and fellows will absorb that experience and try to emulate it in their own careers. And the active component occurs when PIs and other senior-level scientists help students and fellows recognize and build on what they do best.

Crystal Mackall, M.D., also mentors young investigators, specifically the physician-scientists training in the Pediatric Hematology/Oncology Fellowship Program. As Chief of POB, Mackall bears overall responsibility for that program and accepts six new fellows every year. Some, like Shah, gravitate towards clinical research, while others lean more towards basic research. What makes clinical fellows unique, Mackall said, is that despite years spent in medical school and residency, they have little to no experience in research. Already confident and insatiably curious, these fellows must now cultivate a high tolerance for frustration. "Research does not always produce the results you are looking for, and that can be upsetting," Mackall said. "As a mentor and an intellectual guide, my role is to keep fellows stimulated and working. But I also see myself as a coach who reassures them when an experiment does not work out the first time around despite the best of intentions and preparation." Mackall emphasizes that CCR's commitment to training physician-scientists is important, in part, because clinicians have a unique ability to identify shortcomings and research needs in cancer. "We are the largest producer of doctors who are also top-notch scientists," she said. "This is why so many physician-scientists have gone through CCR at some point during their careers."

(Photo: R. Baer)

Crystal Mackall, M.D., (second from right), CCR Clinical Fellows Diana Steffan (left) and Orly Klein (right) examine their patient Jake Schafer in the CCR Pediatric Clinic.

High-School Interns Make the Grade

CCR also remains committed to a much younger group of future scientists and offers opportunities for high school students to get a head start on their research careers. One example is the Werner H. Kirsten Student Internship Program at the NCI laboratories in Frederick, Md. Available to local high school seniors, this program has trained nearly 700 interns since it was created in 1989. Interns work full-time for pay during the summer and then for three hours unpaid every day during the academic year. "Training and developing the next generation of biomedical scientists is a proactive way for CCR to give back to the taxpayers," said Howard Young, Ph.D., Deputy Chief of CCR's Laboratory of Experimental Immunology, who helped launch the program. High-school interns in Young's laboratory extract DNA, and set up sequencing reactions that segregate wild-type mice from mice bred to express inflammatory factors involved in cancer and other diseases. "Colleges love these kids," Young said. "They leave CCR with high-level abilities in gel electrophoresis, restriction enzyme digestion, polymerase chain reaction (PCR), and other analytical methods."

Brittney Reichelt, a senior at Middletown High School in Frederick, and an intern working in Young's laboratory, says the program has also improved her science communication skills. "I am learning how to present my results," she said. "To me, that is really important. And I only knew the bare minimum about PCR when I came here, but now I feel comfortable doing it on my own."

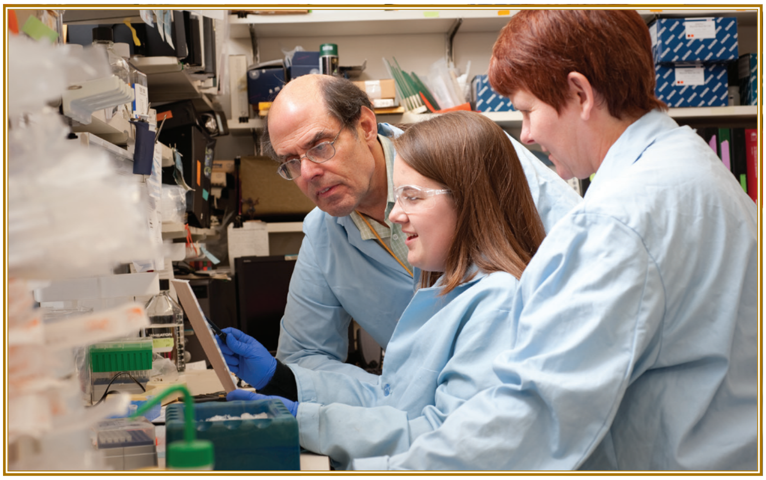
Wilson and Shah also say they are being well prepared at CCR for the careers that await them. Shah, who completed her fellowship last June, plans to stay at CCR to work on new treatments for patients who relapse after receiving bone marrow transplants. Wilson pointed out that CCR's location in Maryland—a hub for biotechnology companies, universities and government health agencies—allows him to network in ways that promote future job prospects. He hopes to transition into a regulatory position working on investigational new drug reviews with the FDA. Ideally, both scientists

> Mentoring is not easy, but it is one of the most important things we do.

will achieve their professional goals and join the ranks of so many who trained at CCR and then met with success in their subsequent careers.

"Mentoring is not easy, but it is one of the most important things we do," Merlino said. "That is why we have an institutional commitment to it."

To learn more about training opportunities, please visit CCR's fellowships and positions Web site at http://ccr.cancer.gov/careers.



(Photo: R. Baer)

Howard Young, Ph.D., high school student trainee, Brittany Reichelt, and Staff Scientist Deborah Hodge, Ph.D., discuss lab results.

Equal Opportunity for Deaf Fellows at CCR

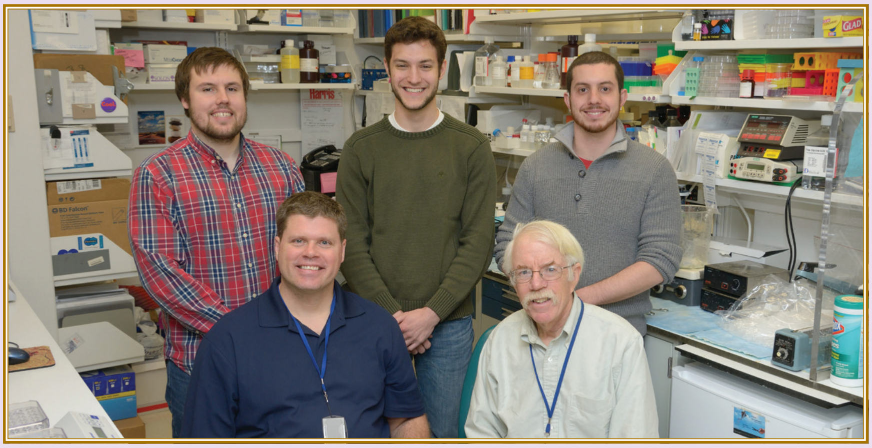
In 1998, Peter Blumberg, Ph.D., recruited his first deaf postbaccalaureate fellow, and he has since continued a commitment to mentoring deaf fellows. Many of them are drawn from Gallaudet University, the leading institution for the deaf and hard-of-hearing, located in Washington, D.C. Over the years, Blumberg's lab technician, Larry Pearce, who is also deaf, and these fellows have co-authored 55 research papers.

"Disabilities are only relevant if they affect skills that you need for a particular task," Blumberg said. "None of my fellows have a disability relevant to science, so they do just fine in the lab."

Blumberg, who taught himself sign language, was inspired to recruit deaf fellows after seeing sign language interpreters at large NIH lectures who had no one for whom to sign. "It seemed like a missed opportunity for the CCR if we did not take advantage of our proximity with Gallaudet," he said. Today, Blumberg has a broad network of contacts at Gallaudet who know his research and who help identify highly qualified candidates interested in the NIH postbaccalaureate fellowship program.

Currently, three deaf post-baccalaureate fellows are working in Blumberg's lab, assisted by a sign language interpreter who facilitates communication with other scientists."Having multiple deaf fellows working together creates an environment in which no one feels like he or she stands out for being different," said Blumberg.

Many of Blumberg's fellows have pursued scientific careers after finishing the two-year program. Several have gone to medical school, others have pursued doctoral research or industry careers, and one has since joined the faculty at Gallaudet.



(Photo: E. Branson)

First row, from left to right: Larry Pearce, Lab Technician, and Peter Blumberg, Ph.D.
Second row: Colin Hill, Timothy Esch, and Ian DeAndrea-Lazarus

To learn more about Dr. Blumberg's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?name=blumberg.

FEATURE

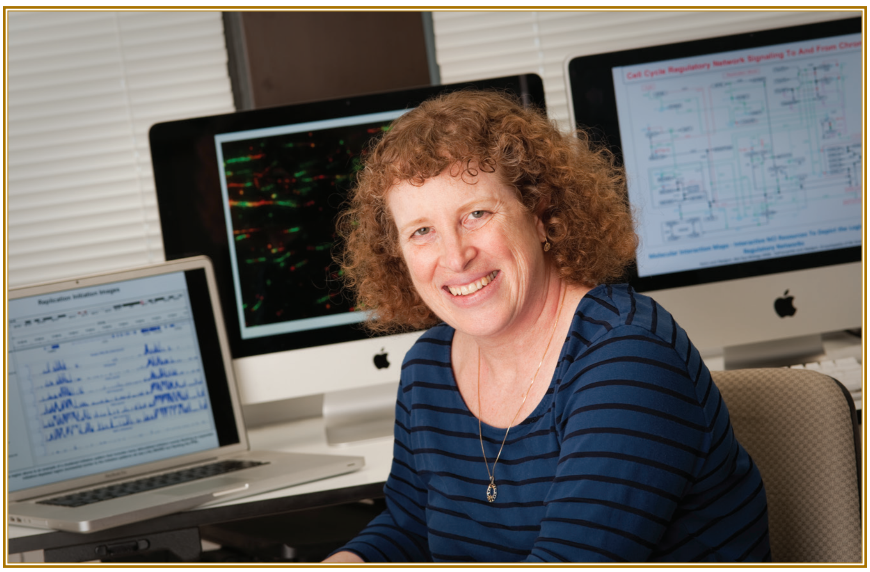
It Starts with a Choice: Cancer Cells and Their Decisions to Replicate

A Senior Investigator in CCR's Laboratory of Molecular Pharmacology (LMP), Mirit Aladjem, Ph.D., has a long-held fascination with the choices cells make when DNA replication goes awry. She realized that the use of alternative signaling pathways lies at the heart of cancer's survival mechanisms—cells that choose to replicate unstable DNA and then divide can seed tumors, while those that choose to self-destruct by apoptosis can impede tumor growth.

Aladjem established her career with research showing that DNA sequences called "replication origins" genetically coordinate replication in mammalian cells. Today, she studies how these intricate signals orchestrate the mysterious process of copying DNA strands. Aladjem's work both advances basic science in cell biology and sets the stage for translational research that can develop therapies to halt the division of malignant cells.

Born and raised in Israel, Aladjem completed her Ph.D. in 1992 at Tel Aviv University, where her studies focused on chemical carcinogenesis. From there, she moved to the Weizmann Institute of Science, in Rehovot, Israel, for a short stint studying protein chemistry, before going to the Salk Institute, in La Jolla, Calif., for a postdoctoral fellowship that set the stage for her research today.

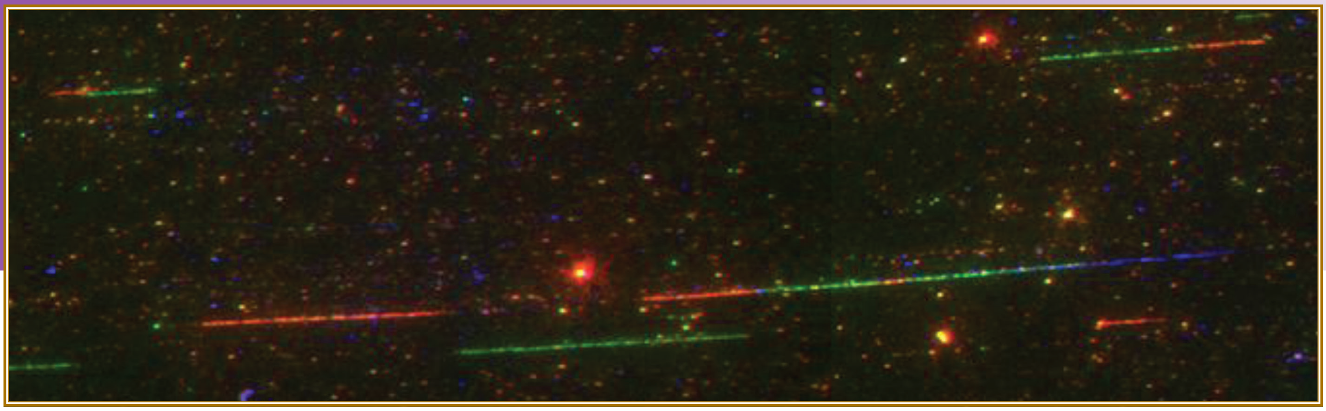
It was at the Salk that Aladjem confronted a contentious dispute in cell biology. On the one hand, many scientists suspected that DNA replication in mammals starts randomly, and not at predetermined genetic sites as had been discovered in yeast cells. Other scientists suspected that DNA replication could not start randomly on chromatin, which is the dense mass of DNA and proteins packed tightly into the nucleus. These researchers held that authentic replication origins in mammalian cells had simply not yet been discovered.



(Photo: R. Baer)

Mirit Aladjem, Ph.D.

Much of what scientists knew at the time about the replication machinery came from studies in yeast. Those investigations had revealed that origins recruit and then bind a suite of proteins known collectively as the "origin recognition complex (ORC)", and that the union of those entities—i.e., the origin, ORC, and some other proteins—creates a "pre-replication complex" that sits on the chromosome and launches replication once activated. A member of the pre-replication complex, known as helicase, starts the replication process when it wedges itself between the helical strands of DNA and splits them



(Image: M. Aladjem, CCR)

DNA replication in human cancer cells. DNA replication can be directly followed by labeling the replicating genome with fluorescent dyes (green and red) and visualizing the DNA strands on microscope slides.

> [. . . human replication origins have direct effects on chromatin and its packaging in the nucleus.]

apart. Each of those strands then becomes a template for newly created DNA.

Through meticulous experimentation in a genomic region called the human beta globin locus (a five-gene cluster involved in the production of hemoglobin, which has been extensively sequenced), Aladjem and her advisor, Geoff Wahl, Ph.D., at the Salk Institute, confirmed for the first time that origins also coordinate replication in mammalian cells. By moving specific sequences around in chromatin, Aladjem and Wahl showed that these origins could also initiate replication at different genomic sites.

Using New Tools to Study Replication Networks

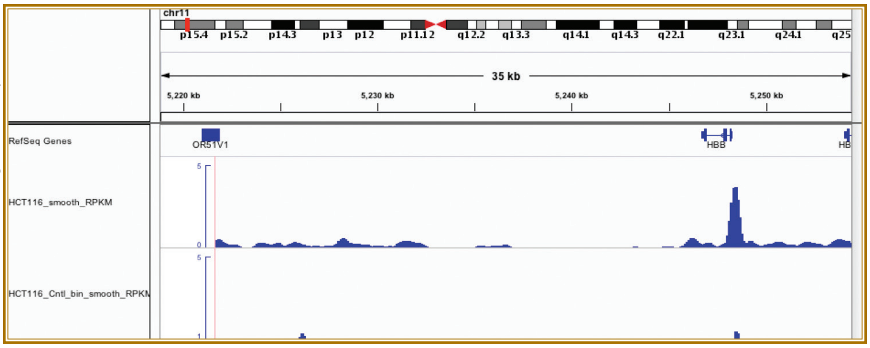
Aladjem came to CCR in 1999, to build on what scientists increasingly recognize as the important role that aberrant DNA replication plays in cancer. Throughout her time here, she has used genetic, biochemical, and bioinformatic tools to study the cell networks that signal to and from chromatin during replication. Part of the challenge, she explains, is to link what scientists glean from studies in yeast with what she and others are learning about replication in mammalian cells. Most of the yeast proteins involved in DNA replication are also encountered in human cells—suggesting their importance because they have been conserved through evolution. However, human origins are more varied than those encountered in yeast, as are the proteins that bind them. Identifying all the mammalian replication proteins and describing the nature of their interactions with chromatin is what drives Aladjem's research today.

At the NCI, Aladjem has shown that human replication origins have direct effects on chromatin and its packaging in the nucleus. Focusing mainly on the human beta globin locus (Aladjem muses that this is her favorite genomic region), she and her research team identified the precise sequences upon which origin activity depends, and then they set out to identify the proteins that bind human origins in sequence-specific ways. Her hope, Aladjem says, is that these proteins facilitate whether replication starts or stops in response to cell cycle signals, which are unregulated in cancer. Should that prove to be true then the aberrant proteins and/or their signaling partners might be targeted with drugs.

But a number of hurdles must still be overcome: Aladjem points out that while she and her colleagues know where origins exist in the human beta globin locus, their locations elsewhere on the chromosome are still being determined. "So we do not know how relevant our findings concerning the human beta globin locus origins will be in other genomic locations," she explained. To broaden their perspective, Aladjem's research team has recently mapped replication origins in whole genome sequences from cancer cells. Mining that database will allow them to study the cell's entire origins population, rather than just a few in the beta globin locus.

Her genomic mapping studies recently showed that human origins differ from those in yeast in an important way. Yeast replication origins are typically dominated by adenine-thymine (AT) sequences, which are more loosely connected to each other than sequences made up of cytosine-guanine (CG) base pairing. The flexibility afforded by the AT bond allows helicase proteins in yeast to come between the DNA strands.

(Image: M. Aladjem, CCR)



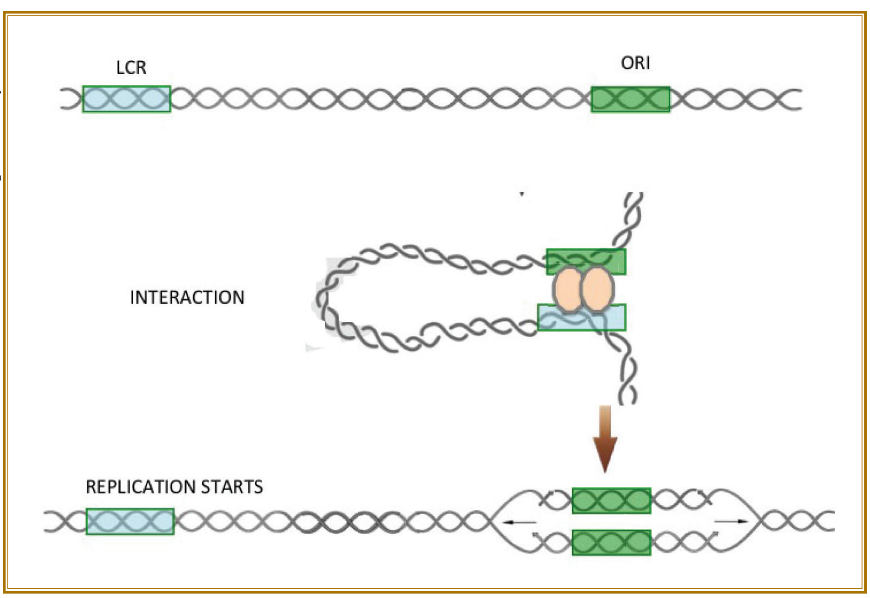
Direct measurement of the locations of replication start sites (replication origins) throughout the whole genome by sequencing short newly replicated DNA. Replication patterns at the beta globin locus on human chromosome 11 (shown at the top track) is shown. Genomic regions that start DNA replication appear as peaks in the bottom track.

The yeast cell's ORC proteins clamp down tightly on AT-rich sequences. But last year in *Genome Research*, Aladjem reported that in mammalian DNA, replication depends on both AT- and CG-rich sequences. Her study showed that replication starts preferentially in methylated CG regions. Methylation is an epigenetic signal that modifies chromatin in a way that might taper transcription and put helicase into action.

That same study also revealed that DNA replication occurs in areas with low levels of gene transcription, but where gene transcription levels are high, DNA replication is diminished. That finding addressed another ongoing debate in the field: previously, some scientists had claimed that replication represses transcription, while others had reported that transcription and replication occur hand-in-hand. "We found that everyone is right," Aladjem said. "When transcription goes from zero to low levels, it encourages replication; but when transcription goes from low levels to high, it prevents replication." Aladjem speculates that this occurs because both processes require large protein complexes—the pre-replication complex and the transcription initiation complex—which compete for space on chromatin. "These complexes do not like to sit next to each other," she explained.

Biological activity at replication origins ultimately distills to whether cells will replicate DNA and pass through checkpoints that govern cell division. This is vitally important in the context of cancer. When confronted with potentially harmful mutations, or with conditions that might mutate replicating DNA, cells typically arrest at the early (G1) phase of the cell cycle—before replication occurs—or they pause in the middle of synthesis (called S-phase) until the DNA damage or conditions that might cause it are addressed. "Cancer cells do not exhibit the same active cell-cycle checkpoint pathways found in noncancerous cells," Aladjem said. "So we study how cell-cycle regulatory pathways interact with chromatin to activate checkpoints when needed, and how these interactions vary in cancer cells."

(Image: M. Aladjem, CCR)

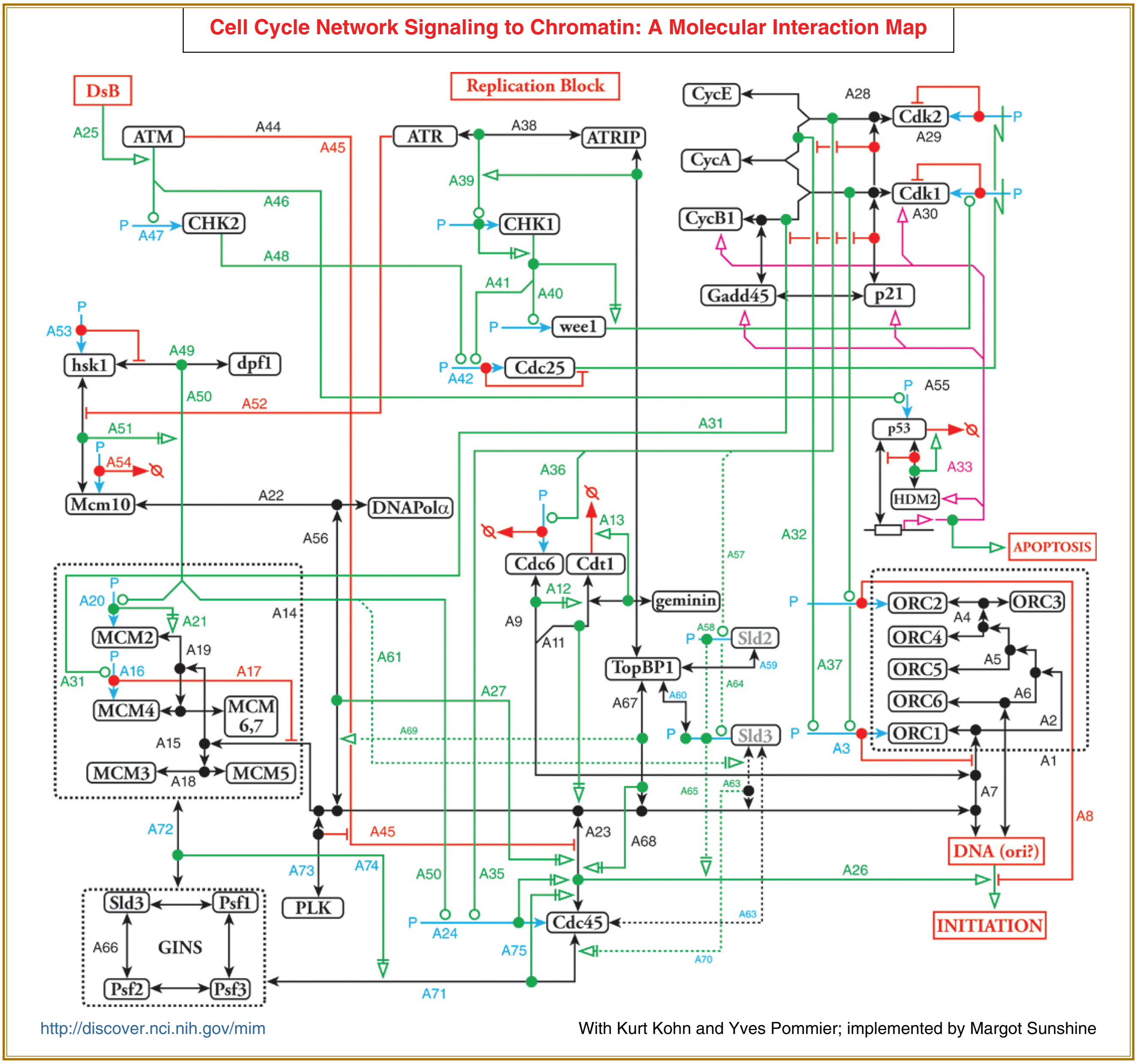


The locations of replication origins are determined by protein complexes that promote interactions between distant regions on chromatin.

Replication as a Target in Drug Development

Now, those investigations are generating translational opportunities because interfering with replication offers a new mechanism for cancer therapy. Yves Pommier, M.D., Ph.D., Chief of LMP, collaborates with Aladjem on applications for her research in drug development. "If we can find differences in how cancer and normal cells replicate DNA, and find fragile points in the cancer cell's replication initiation program, then we can rationalize therapeutic approaches to selectively kill these cells," he said.

Pommier's research has shown that a class of drugs that inhibit topoisomerase (an enzyme that



(Image: M. Aladjem, CCR)

A molecular interaction map of the events leading to replication. For details, see http://discover.nci.nih.gov/mim

regulates the over- or under-winding of DNA) has a "strong effect" on replication origins. These effects are especially pronounced in cancer cells. Through his collaborations with Aladjem, Pommier found that cancer cells have trouble adjusting replication programs in response to topoisomerase inhibitors, so they undergo apoptosis, while normal cells simply pause during division until the drug washes out from the cells. "Dr. Aladjem has the molecular biology know-how we need for this research, while my laboratory has more expertise on the drug side; this is how our work is complementary," he said.

Basic discoveries made by the Aladjem team are beginning to reap rewards for translational research. As Aladjem explains, "Our findings about human replication origins are prompting scientists to rethink replication complexes as determinants of cell-growth responses; this is becoming more and more important to the development of new targeted therapies. The scientific community has begun to unravel the mystery of mammalian replication origins."

To learn more about Dr. Aladjem's research, please visit her CCR Web site at http://ccr.cancer.gov/staff/staff.asp?name=aladjem.

Tackling Drug Resistance Mechanisms in the Stroma

Michael Ostrowski, Ph.D., spent five years at NCI, working with Edward Skolnick, M.D., formerly Chief of the Laboratory of Tumor Virus Genetics (LTVG), and Gordon Hager, Ph.D., now Chief of CCR's Laboratory of Receptor Biology and Gene Expression. Ostrowski is currently Professor and Chair of the Department of Molecular and Cellular Biochemistry at The Ohio State University Medical Center (OSUMC), in Columbus. He is also Co-Director of OSUMC's Comprehensive Cancer Center Program in Molecular Biology & Cancer Genetics.

Death rates for many cancers have fallen during the last several decades, but in some cases, that trend is starting to level off. The death rate for breast cancer, for instance, fell by roughly 25 percent between 1990 and 2010, reflecting advances in targeted therapies such as tomaxifen for estrogen-receptor positive tumors. However, 30-40 percent of breast cancer patients treated with tamoxifen will become resistant to it, and this amounts to a very large number of people for whom we do not have many other treatment options. So while targeted therapies have led to some important successes, we still also have to address the resistance problem. One approach is to address resistance mechanisms specifically within cancer cells. However, in my laboratory, we take a complementary focus upon the tumor stroma as well, meaning the noncancerous fibroblasts, endothelial cells, and immune cells in the tumor's microenvironment.

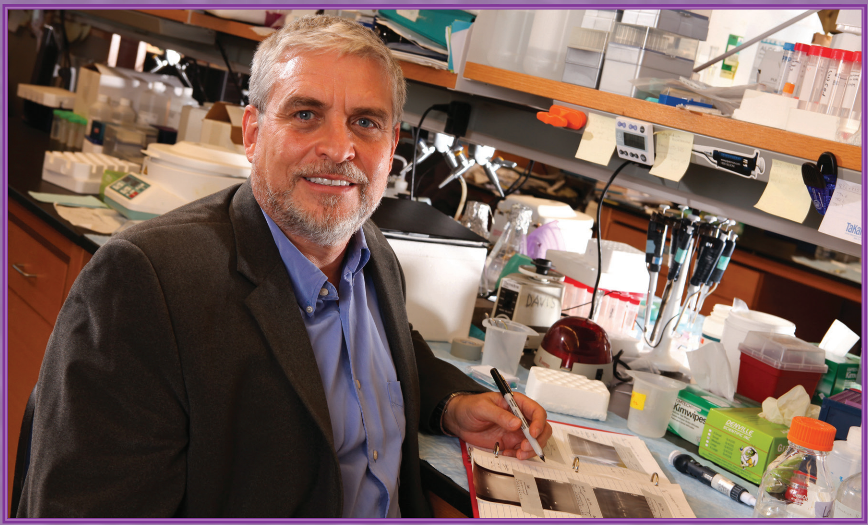
Signaling between stromal cells and tumor cells can drive cancer progression and metastatic spread. My collaborator Gustavo Leone, Ph.D., also from Ohio State University, and I were the first to show that deleting the tumor suppressor PTEN from stromal fibroblasts accelerates angiogenesis and metastasis in a mouse model of breast cancer. PTEN normally suppresses a signaling pathway involving PI3-kinase and a downstream transcription factor known as Ets2, which contributes to cancer progression. Our work showed that deleting PTEN from stromal fibroblasts activates Ets2, and that this makes tumors much more aggressive.

> So while targeted therapies have led to some important successes, we still also have to address the resistance problem.

When I came to NCI as a Staff Fellow in 1980, the cloning revolution was just getting under way. In the Hager lab, we cloned the mouse mammary tumor virus and used it to study how glucocorticoids regulate gene expression. These were exciting times to be at NCI as so much was happening in science, and in cancer-related research. NCI was a great training environment, and I had a very productive experience there. Many of the fellows I worked with there have gone on to great careers of their own, and I still collaborate with some of them today. For instance, I collaborate on an NCI-supported Program Project Grant with Morag Park, Ph.D., now at McGill University, who was a postdoctoral fellow working with George Vande Woude, Ph.D.

I also co-direct one of the six scientific programs in our NCI-Designated Comprehensive Cancer Center at OSUMC. What is great about the Center is that it unites faculty from throughout Ohio State, which is a huge university with 16 colleges. We have members from 14 of those colleges,

(Photo: Courtesy of M. Ostrowski)



Michael Ostrowski, Ph.D.

Many of the fellows I worked with there have gone on to great careers of their own, and I still collaborate with some of them today.

representing a diversity of expertise, from engineering to agriculture to veterinary medicine and even to the humanities, all connected by a focus on cancer.

The Cancer Center also facilitates translational opportunities, and now my laboratory has begun to investigate how stromal PTEN expression correlates with treatment outcomes in human patients. We know that about 30 percent of all human breast cancer patients express PTEN at low levels that trigger Ets2 activation. So we participate in clinical trials that stratify tumors according to PTEN expression, to see if low levels also predict more frequent treatment failures. Targeted treatment with Herceptin (trastuzumab) or lapatinib only works in about half of all HER2-positive breast cancers, and we think that the nonresponders may have deficient PTEN in the stroma. We also know that loss of PTEN and Ets2 activation via PI3 kinase results in the production of secreted factors with an influence on tumor growth: tumors grow faster, become more vascularized, exhibit more inflammation, and are more resistant to therapy. Our hypothesis is that we can address that deficiency with small molecule inhibitors directed at the PI3-kinase pathway, and that this might break the resistance against Herceptin or lapatinib therapy.

I still see myself as a basic scientist, but with increasing opportunities to move what we do in the laboratory towards the clinic. Our Cancer Center facilitates this translational direction—we identify signaling pathways in humanized mouse models and then we investigate those pathways in human tumors by working back and forth to develop and test hypotheses. Using this approach, we identified more than 200 secreted factors in PTEN-deficient mice, and now we are looking at how the expression of those factors correlates with therapeutic responses in human patients.

We are also starting to work in the area of pancreatic cancer, which is a terrible disease with a huge stromal component. For this cancer, it can be difficult to isolate tumor cells from the stroma, because there is a high influx of immune cells and a lot of leaky blood vessels. So the translational aspect of our work focuses upon attacking resistance to therapy. And we direct our efforts at the tumor stroma because the cells are genetically stable and, therefore, may be amenable to more durable therapies for which resistance does not pose as much of a challenge.

The stage is set for stromal research to contribute significantly to the survival of cancer patients over the next decade, and I'm excited to be a part of this effort.

I still see myself as a basic scientist, but with increasing opportunities to move what we do in the laboratory towards the clinic.

CCR connections is available online at http://home.ccr.cancer.gov/Connections

Web Sites with More Information about CCR

Center for Cancer Research
http://ccr.cancer.gov

Office of the Director
http://ccr.cancer.gov/about/OfficeDirector.aspx

Our News
http://ccr.cancer.gov/news

Office of Training and Education
http://ccr.cancer.gov/careers/OfficeEducation.aspx

Patient Information on Cancer and Clinical Trials

Open NCI Clinical Trials
http://www.cancer.gov/clinicaltrials/search

How to Refer a Patient
http://bethesdatrials.cancer.gov/health-care-professionals/index.aspx

NCI Cancer Information Service
http://www.cancer.gov/aboutnci/cis
1-800-4-CANCER (1-800-422-6237)

Understanding Cancer Series
http://www.cancer.gov/cancertopics/understandingcancer

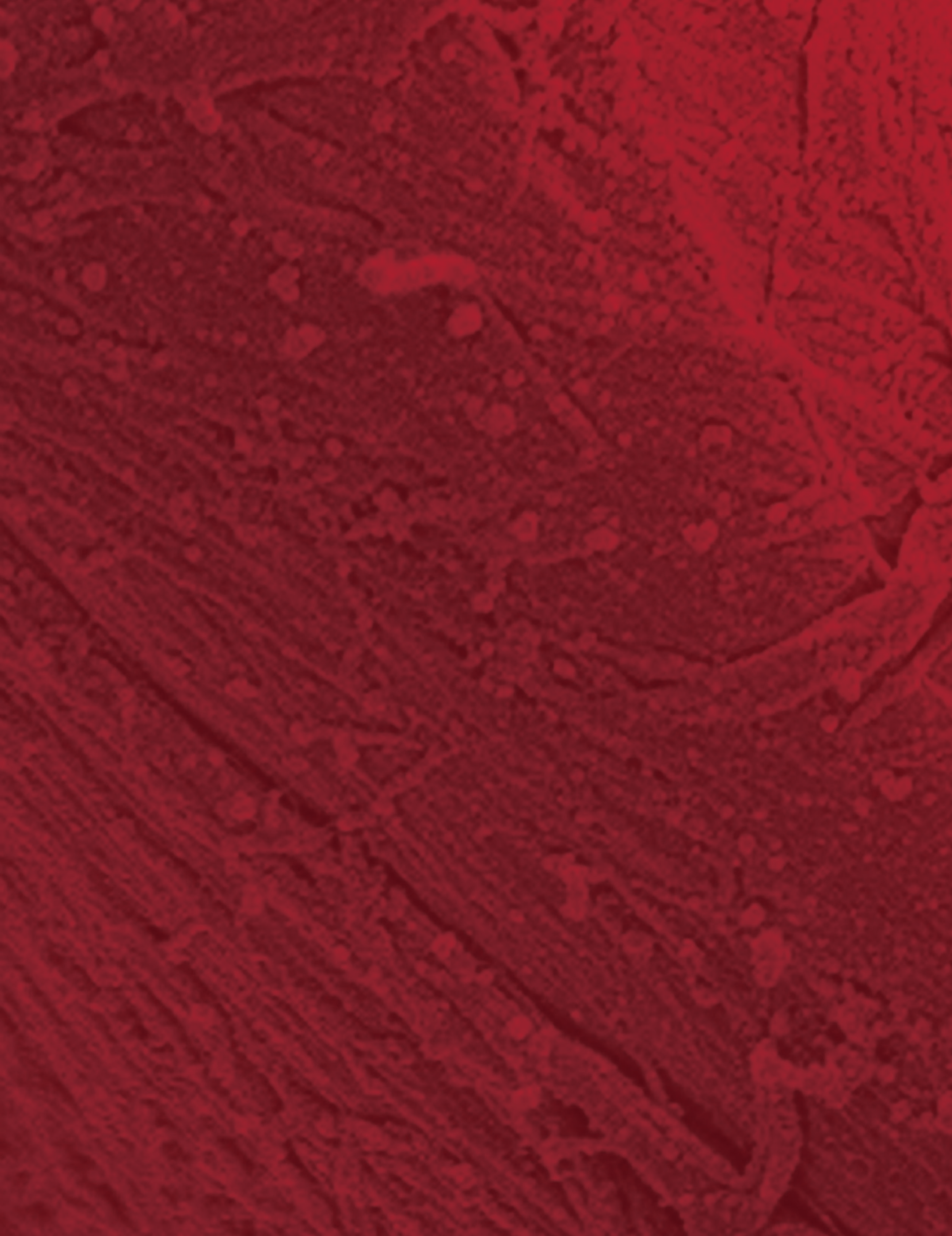
CCR Clinical Cancer Trials in Bethesda, MD
http://bethesdatrials.cancer.gov

Additional Links

National Cancer Institute (NCI)
http://www.cancer.gov

Working at NCI
http://www.cancer.gov/aboutnci/working

National Institutes of Health (NIH)
http://www.nih.gov



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